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Biological Nutrient Removal: Minimizing Carbon and Oxygen Requirements

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in Chemical and Biochemical Engineering

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Abstract

With increasing focus on the carbon footprint of wastewater treatment and rapidly emerging paradigm shift towards resource recovery, energy consumption minimization and utilization of readily available organics for biological nutrient removal in municipal wastewater treatment plants is eliciting significant interest. The objective of this PhD work is to investigate non-traditional approach to minimize carbon and energy demand for biological nutrient removal

The feasibility of using thermal alkaline treated municipal wastewater biosolids as an alternative carbon source for biological phosphorus removal was investigated. Two sequencing batch reactors (SBRs) were operated with synthetic volatile fatty acids (acetic acid and propionic acid) and readily biodegradable organics produced from the alkaline hydrolysis of municipal wastewater biosolids (Lystek) as the carbon source, respectively. Municipal wastewaters with different strengths and COD:N:P ratios were tested. The reactors' performances were found to be comparable with respect to nitrogen and phosphorus removal. It was observed that phosphorus removal efficiencies were between 98% to 99% and 90% to 97% and nitrogen removal efficiencies were 78% to 81%, and 67% for the SynVFA and Lystek, respectively. However, the kinetics for phosphorus release and uptake during the anaerobic and aerobic stages with Lystek were observed to be significantly lower than SynVFA due to the presence of higher order VFAs (C4 and above) and other fermentable organics in the Lystek.

A novel integrated partial nitrification-denitrifying phosphorus removal system enriched with non-conventional phosphorus accumulating organisms (PAOs) was developed for treating carbon limited synthetic wastewater. Atypical operating conditions, such as low DO (0.3 ± 0.05 mg/L) and relatively long solid retention time (SRT) of 15 days, favored the enrichment of a wide variety of denitrifying phosphorus accumulating organisms (DPAOs), such as *Rhodocyclus*, *Dechloromonas*, and *Cytophaga*. In contrast to the *Accumulibacter*, these microorganisms can sustain in a very low DO environments and simultaneously perform denitrification and enhanced biological phosphorus removal (EBPR) using oxygen, nitrite, and nitrate as electron acceptors. Fermentative microorganisms, such as *Bacteroidetes*, were also observed. Low DO also favored the washout of nitrite oxidizing bacteria (NOB), leading to simultaneous partial nitrification-denitrifying phosphorus removal

(PNDPR). Partial nitrification at low DO also facilitated the washout of glycogen accumulating organisms (GAOs) from the PNDPR system. When operated with synthetic wastewater, stable operating conditions were achieved within 3-4 SRT turnovers and simultaneous nitrification-denitrification (SND), nitrogen, and phosphorus removal efficiencies were maintained above 90%. Of the total P removed by EBPR, P-removal percentages via nitrite, nitrate, and oxygen were 69%, 23%, and 8%, respectively. Utilizing nitrite instead of nitrate and low DO aeration implies a significant reduction in carbon and aeration requirement for simultaneous denitrification and phosphorus removal.

Lastly, the PNDPR system was implemented for treating real municipal wastewater with low COD/N ratio. In addition to low DO (0.3 ± 0.05) mg/L, an extended anaerobic contact time facilitated the efficient utilization of organic carbon in wastewater and nutrient removal without carbon supplementation. Low DO during the aerobic stage was favorable for anoxic P-removal rather than aerobic as evidenced by simultaneous N and P removal in the cyclic test. Most of the rapid initial P uptake during the aerobic phase was attributed to DPAOs utilizing nitrites rather than nitrates, with $\text{NO}_x\text{-N}$ accumulating after almost complete utilization of the stored PHA and associated P uptake. The ratio of COD utilized to $\text{NO}_x\text{-N}$ reduced was estimated to be 4.2, which also implies efficient utilization of carbon for nutrient removal. Due to the integration of nitrification with denitrifying phosphorus removal, more than 70% N-removal and 90% P-removal was observed even at low COD/N ratio of 5. COD removal was not impacted by low DO as effluent sCOD concentrations were consistently below 25 mg/L. Compared to the conventional EBPR process, the low DO-SNDPR process implies maximum reductions in energy and carbon consumption of 35% and 45%, respectively. This can significantly reduce the overall carbon footprint of municipal wastewater treatment plants.

Keywords

Alternative carbon source; enhanced biological phosphorus removal; phosphorus accumulating organisms; simultaneous N and P removal; carbon deficient municipal wastewater; low aeration demand; nitrite-shunt

Summary for Lay Audience

Nutrients in wastewater effluents, i.e. nitrogen (N) and phosphorus (P) have elicited significant interest because of eutrophication of lakes and rivers in North America and many other parts of the world. Eutrophication is generally defined as the enrichment of N & P leading to the uncontrolled growth of aquatic plants/planktons, resulting in low dissolved oxygen (DO), murky water, and destruction of the diversity of aquatic species.

In biological wastewater treatment process, nutrients are removed by bacterial microorganism consuming N and P from the wastewater for their microbial growth and maintenance.

While wastewater treatment plants (WWTPs) are vital for the safety of public health and environment, they are also one of the largest scavengers of material and energy in the community. The energy consumption by wastewater treatment plants account for 0.25%-1% of the national energy consumption in many countries. This consumption is expected to increase with increasing population, economic activity, stricter regulations, and infrastructure ageing. Furthermore, to enhance the performance of BNR, readily biodegradable carbon is generally added, if the raw wastewater does not contain enough readily biodegradable carbon. Typically, acetic acid and propionic acid are used as a carbon source, which significantly increases operational costs. Besides the economic aspects, excessive use of these chemicals also increases the carbon footprint of the WWTPs.

This PhD project aimed at developing strategies for resource recovery and minimizing carbon and energy consumption in WWTPs.

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Co-Authorship Statement

The content of this PhD thesis is either published or under review as listed below:

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List of Abbreviations and Symbols

WWTP	Wastewater treatment plant
USEPA	United states environmental protection agency
COD	Chemical oxygen demand
BOD	Biological oxygen demand
SNDPR	Simultaneous nitrification-denitrification and phosphorus removal
DPAO	Denitrifying phosphorus accumulating organism
PAO	phosphorus accumulating organism
GAO	Glycogen accumulating organism
BNR	Biological nutrient removal
EBPR	Enhanced biological phosphorus removal
VFA	Volatile fatty acids
TSS	Total suspended solids
VSS	Volatile suspended solids
TP	Total phosphorus
SP	Soluble phosphorus
TN	Total nitrogen
SN	Soluble nitrogen
AOB	Ammonium oxidizing bacteria
NOB	Nitrite oxidizing bacteria
DO	Dissolved oxygen
SND	Simultaneous nitrification and denitrification
PHA	Poly-hydroxyalkanoate
PHB	poly-hydroxybutyrate
PHV	poly-hydroxyvalerate
PH2MV	poly-methylvalerate
DS	Dry solids
HTO	Hydrothermal oxidation
THP	Thermal hydrolysis process
OHO	Ordinary heterotrophic organisms
WAS	Waste activated sludge
PS	Primary sludge
CG	Crude glycerol
TKN	Total Kjeldahl Nitrogen
CE	Clarified effluent
SBR	Sequencing batch reactor
LysVFA	Lystek volatile fatty acids
SynVFA	Synthetic volatile fatty acids
SPRR	Specific phosphorus release rate
SPUR	Specific phosphorus uptake rate
SDNR	Specific denitrification rate
PNDPR	Partial nitrification denitrifying phosphorus removal
PLC	Programmable logic controller
SRT	Solid retention time
HRT	Hydraulic retention time

ANAMMOX	anaerobic ammonium oxidation
ffCOD	Flocculated and filtered COD
RAS	Recycled activated sludge
bsCOD	Biodegradable soluble COD
Y_n	Net biomass yield
q_{PHA}	Acetate uptake rate constant
q_{PP}	Polyphosphate storage rate constant
μ_{PAO}	Maximum growth rate of PAOs
K_{PHA}	Saturation coefficient for PHA in PAOs
Y_{PO_4-P}	Yield of Poly-P required per PHA storage
Y_H	Yield of heterotrophic organism growth
Y_{PAO}	Yield of PAOs growth
Y_{obs}	Observed biomass yield
μ_{max}	maximum specific growth rate
K_o	half-velocity coefficient
b	specific endogenous decay coefficient
θ	temperature correction coefficient
NAR	Nitrite accumulation ratio
ACR	Ammonia conversion ratio

Chapter 1

Introduction

1. Rationale

Nutrient (N & P) enrichment of waterbodies have elicited significant global interest because of eutrophication in lakes and rivers. Eutrophication, i.e., uncontrolled growth of algal biomass reduces the dissolved oxygen level, thereby, significantly impacting the aquatic life and our ecosystem. Excessive nitrate in drinking water is primarily responsible for methemoglobinemia (also known as blue baby syndrome) in infants under the age of 6 months and pregnant women. According to the USEPA, nearly 25% of the water body impairments are caused by nutrient-related issues (USEPA, 2007). In order to minimize the extent of nutrient impairments from point sources, stricter jurisdictional regulations have been imposed to reduce N and P discharge limits, for example the typical discharge limits in North America for phosphorus and nitrogen are 0.5 to 1 mg/L and 3 to 10 mg/L, respectively (Oleszkiewicz et al., 2015; Oleszkiewicz & Barnard, 2006). Due to the stricter discharge limits, municipalities are challenged with finding environmentally sustainable and cost-effective nutrient removal processes. Even though traditional/first generation biological nutrient removal (BNR) processes are capable of achieving low effluent nitrogen and phosphorus concentrations, the process configurations are not generally configured for carbon and energy efficiency. This led to the development of 2nd generation BNR processes to enhance process intensification via reduction of operational cost, waste generation, improvement of resource recovery in the form of organic carbon and bioenergy, and reduction of overall carbon footprint (Gao et al., 2017; He et al., 2016; Li et al., 2019; Roots et al., 2020; Wang et al., 2015; Yang et al., 2016).

Although wastewater treatment plants (WWTPs) are vital for public health and environmental protection, they are also one of the largest scavengers of material and energy in the community. The energy consumption by wastewater treatment plants account for 0.25%-1% of the national energy consumption in many countries (Gu et al., 2017). This consumption is expected to increase with increasing population, economic activity, stricter regulations, and infrastructure ageing (Mo & Zhang, 2013). Furthermore, to enhance the performance of BNR, external carbon is generally required (Shen & Zhou, 2016) for organics-limited wastewaters. Typically, synthetic carbon sources, such as methanol, glycerol, and acetic acid are used, which significantly increase operational

costs and carbon footprint of WWTPs. Recently, wastewater treatment plants have strived to recover resources including internal carbon for use in biological phosphorous removal. Thermal treatment of digested and/or undigested biomass is being considered as the forefront of technologies for carbon recovery in municipal wastewater treatment plants (Cano et al., 2015; Pilli et al., 2015). Recently, a relatively low-temperature thermal-alkaline hydrolysis process (Lystek®) has been reported as an emerging technology for solubilization of readily biodegradable carbon of raw as well as digested sludge (Singh et al., 2016). The Lystek product was reported not only to have 40%-50% of COD as soluble COD (sCOD) and an order of magnitude higher VFAs (10-15 g/L) compared to traditional biosolids treatment processes but also higher N, P, and suspended solids. COD solubilization and VFA concentrations vary depending on the source of solids within the WWTP or from other source. In order to successfully integrate Lystek® into BNR processes, it is important to investigate the impact of carbon diversion via Lystek process into the mainstream wastewater treatment.

Simultaneous nitrification-denitrification and phosphorus removal (SNDPR) has emerged as a promising alternative to traditional BNR for minimizing carbon and energy requirements. Numerous bench scale and two full-scale studies showed the potential for significant carbon and energy savings in SNDPR process (Bassin et al., 2011; He et al., 2016; Li et al., 2019; Roots et al., 2020; Wang et al., 2015; Yang et al., 2016). An ideal SNDPR system would maximize carbon saving via linking denitrification with phosphorus removal and should incorporate denitrifying phosphorus removing microorganisms (DPAOs) in the simultaneous denitrification and phosphorus removal mechanism. Even though some studies with synthetic wastewater were able to successfully integrate DPAOs in the overall microbial community, all the SNDPR studies with real wastewater failed to achieve DPAOs enrichment due to operating at high DO. Therefore, the SNDPR was limited to denitrification via ordinary heterotrophs and P removal was primarily performed by PAOs using O₂ as an electron acceptor. DPAO enrichment in the SNDPR biomass will significantly reduce carbon consumption since the same internal carbon will be used for simultaneous N and P removal. In addition, when denitrifying P-removal is integrated with partial nitrification i.e. conversion of

ammonia to nitrites rather than nitrates, it will further reduce both carbon and energy consumption.

1.2. Research Objectives

The overall goal of this PhD thesis is to investigate strategies for minimizing carbon supplementation and energy consumption in biological nutrient removal process. The specific objectives are outlined below:

To investigate the effectiveness of the low temperature thermo-alkaline hydrolysis (Lystek®) process (Temperature: 70-75°C, pH: 9.5-10, pressure: 1 atm) treated municipal biosolids as an alternative carbon source in enhanced biological phosphorus removal (EBPR)

To study the impact of dissolved oxygen concentration and DPAOs: nitrifiers population ratio on nutrient removal in EBPR process

To investigate simultaneous partial nitrification-denitrifying phosphorus (PNDPR) removal with synthetic wastewater at low COD/N ratio using enriched DPAO cultures

To investigate simultaneous nitrification-denitrifying phosphorus removal (SNDPR) at low DO for treating carbon-limited municipal wastewater

1.3. Thesis Organization

Chapter 1 provides a brief overview and motivation behind this PhD project. It briefly summarizes the most relevant literature and knowledge gaps and emphasize the need for this research.

In chapter 2, a comprehensive literature review of biological nutrient removal is presented. It discusses pertinent wastewater characteristics and fundamental N and P removal mechanisms in both traditional and 2nd generation BNR. It also outlines the current knowledge gaps and scope of further research.

Chapter 3 is a published research paper entitled “Enhanced Biological Phosphorus Removal Using Thermal Alkaline Hydrolyzed Municipal Wastewater Biosolids”. The aim of this study was to evaluate the potential of municipal biosolids

treated by Lystek® process as a source of carbon for enhanced biological phosphorus removal from municipal wastewater. A control reactor with synthetic supplemental carbon source was also operated and its performance was compared with the biosolids fed reactor. In-line soluble phosphorus concentrations were measured in order to evaluate the kinetics of phosphorus transformation.

Chapter 4 is also a published research article entitled “Impact of Dissolved Oxygen Concentration and DPAOs: Nitrifiers Population Ratio on Nutrient Removal in EBPR Process”. This study investigated the impact of low dissolved oxygen concentration and DPAOs to nitrifiers population ratio on nutrient removal. The DPAOs enrichment process was carried out in a separate SBR, capable of utilizing both NO₂-N and O₂ as an electron acceptor. NOB washout from the nitrifying sludge was obtained in a separate SBR operated under low DO (0.3-0.5 mg/L) condition.

Chapter 5 is a research paper, currently under review, entitled “Partial Nitrification-Denitrifying Phosphorus Removal (PNDPR) For Energy and Carbon Minimization”. This study investigated a BNR system using anaerobic-aerobic SBR integrating partial nitrification-denitrifying P-removal for carbon and energy-efficient N and P removal. The unique feature of the SBR was very low DO (0.3±0.05 mg/L) and low COD/N ratio (4 mg COD/mg N). Several batch studies were conducted to elucidate the pathways for N and P-removal. This study also investigated the relative abundance of various microorganisms and their role in the PNDPR system.

Chapter 6 is also a research paper entitled “Simultaneous Nitrification-Denitrifying Phosphorus Removal (SNDPR) at low DO for treating carbon-limited municipal wastewater”. This study demonstrated a single sludge SNDPR process removing C, N, and P from real municipal wastewater. The wastewater COD/N ratio varied between 5-10, representing a challenging environment for simultaneous N and P removal. In contrast to prior studies on full-scale SNDPR, simultaneous denitrification and P-removal was primarily carried out via DPAOs. No carbon supplementation was provided throughout the study.

Chapter 7 summarizes major knowledge contributions as an outcome of this research. It also includes some recommendation for future research.

1.4. Thesis Format

This thesis has been prepared in the integrated-article format according to the specifications provided by the School of Graduate and Postdoctoral Studies located at the Western University. Chapter 3 of this thesis has been published in Journal of Environmental Sciences. Chapter 4 of this thesis has been published in International Journal of Environmental Science and Development. Chapter 5 is currently under peer review in the Journal of Environmental Sciences. Chapter 6 is currently under peer review in Science of the Total Environment. Each chapter includes its own introduction and references. As far as possible, uniform and standard symbols are used throughout the thesis.

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Chapter 2

Literature Review

1. Background

Phosphorous is one of the vital elements of life besides carbon, hydrogen, oxygen, and nitrogen on this earth. It is an integral part of the cell structure including : cell membrane, genetic materials, and in the skeleton of all vertebrates (Filippelli, 2008). Growing global population and improved living standards is creating tremendous pressure on preserving water quality. Enrichment of nutrients i.e. nitrogen and phosphorous in the water bodies has been considered as a topic of interest over a long time and now become more prominent because of eutrophication in lakes, rivers, and coastal water, resulting in reduced dissolved oxygen concentration and adversely impacting aquatic life.

While both nutrients are essential for excessive production of aquatic plants, phosphorous is considered as the true limiting component since nitrogen is never limiting due to the activity of nitrogen fixing bacteria. Therefore, phosphorous reduction has been primarily considered as a control strategy for eutrophication.

Phosphorus can enter into the water bodies from various point sources, such as industrial and municipal wastewater, and non-point sources such as agricultural run-off . While the non-point sources are difficult to control, phosphorous in the industrial and municipal wastewater can be effectively controlled through treatment process.

Enhanced biological phosphorus removal (EBPR) is a sustainable and environmentally friendly engineered wastewater treatment process that is capable of achieving low effluent phosphorus concentrations and should be considered as the first line of defense for phosphorus reduction. EBPR was pioneered by James Barnard in the early 1970's when he observed enhanced biological phosphorus removal in a pilot scale nitrogen removal plant where the activated sludge was subjected to sequencing anaerobic-aerobic zones in which the anaerobic zone was completely free of nitrates and dissolved oxygen (Barnard, 1975). The findings led to the development of the Phoredox and Bardenpho process configurations, which are the basis of all biological nutrient removal processes today (Barnard, 2006; Barnard, 1975).

Even though the first generation BNR processes are capable of achieving low effluent nutrient concentration, the process configurations are not generally configured for carbon and energy efficiency. This led to the development of 2nd generation BNR

processes to enhance process intensification via reduction of process footprint, operational costs, waste generation, improvement of resource recovery in the form of organic carbon and bioenergy, and reduction of greenhouse gas emissions (N_2O , CO_2 , etc.) (Gao *et al.*, 2017; He *et al.*, 2016; Li *et al.*, 2019; Roots *et al.*, 2020; Wang *et al.*, 2015; Yang *et al.*, 2016).

2 Municipal Wastewater Characteristics

2.1. Organics

Organic compounds that are typically found in municipal wastewater consist of carbohydrates, protein, fats, grease, lignin, detergents, and their degradation products. Traditionally, organic compounds in municipal wastewater are measured as biological oxygen demand (BOD) or chemical oxygen demand (COD). Figure 2.1 shows the fractionations of COD in wastewater (Metcalf and Eddy, 2014).

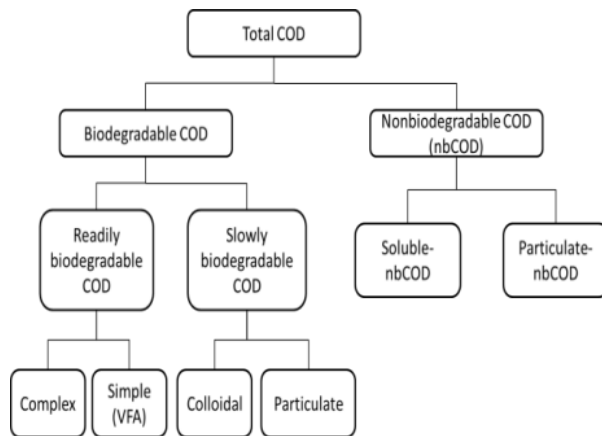


Figure 2.1 Fractionation of COD in wastewater(Metcalf and Eddy, 2014)

2.2 Solids

Solids in municipal wastewater is a critical parameter for designing both liquid and solid treatment trains. Solids are typically composed of floating, settleable, colloidal, and soluble materials. Fig.2.2 shows the interrelationships of solids in wastewater. Suspended solids refer to the portion of total solids retained on a $1.2\mu\text{m}$ filter paper after being dried at 105°C . The total dissolved solids consist of particulate/colloidal ($0.001\mu\text{m}$ to $1.2\mu\text{m}$) and soluble solids ($<0.45\mu\text{m}$). The VSS/TSS ratio in municipal wastewater typically ranges from 0.6-0.8 (Henze *et al.*, 2008; Metcalf and Eddy, 2014; WEF, 2005).

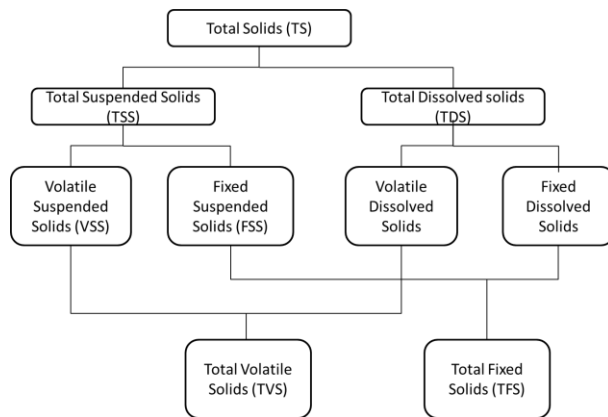


Figure 2.2 Interrelationship of solids found in wastewater (Metcalf and Eddy, 2014)

2.3 Nitrogen

The most common forms of nitrogen in municipal wastewater are: $\text{NH}_3\text{-N}$, $\text{NH}_4^+\text{-N}$, N_2 , $\text{NO}_3\text{-N}$, $\text{NO}_2\text{-N}$, and organic nitrogen (Metcalf and Eddy, 2014). Fig.2.3 shows the fractionation of nitrogen in municipal wastewater. Total Kjeldahl nitrogen (TKN) usually consist of 60% ammonia and 40% organic nitrogen (WEF, 2005).

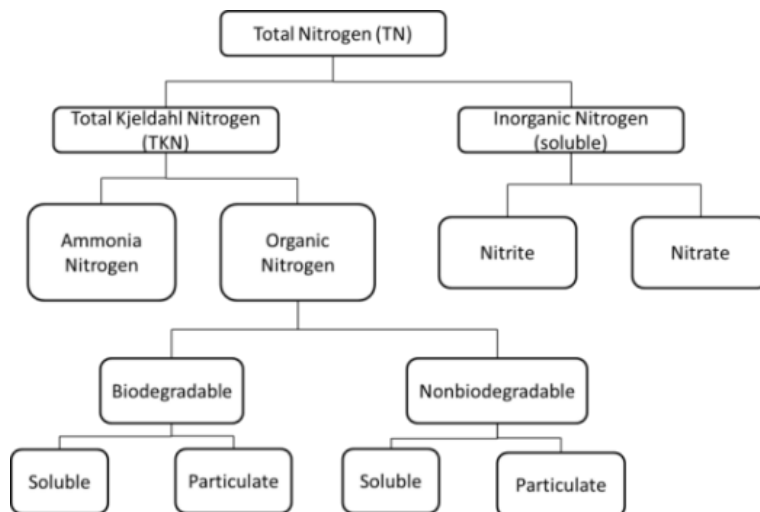


Figure 2.3 Nitrogen fractionation in wastewater (Metcalf and Eddy, 2014)

2.4. Phosphorus

The most common forms of phosphorus in municipal wastewater are: (1) orthophosphates (PO_4^{3-} , HPO_4^{2-} , H_2PO_4^- , H_3PO_4), (2) poly-phosphates, (3) organic phosphate. The fractionation of phosphorus in wastewater is shown in Table 2.1. About

50% of the influent phosphorus is found as orthophosphate. Most of the polyphosphate and organic phosphate are generally hydrolyzed to orthophosphate during the biological treatment and can be easily assimilated by microorganism and/or chemically precipitated (Curtin, 2011).

Table 2.1 Typical forms of phosphorus in municipal wastewater (Curtin et al., 2011)

Phosphorus form	Typical concentrations (mg/L)
Orthophosphate	3-4
Polyphosphates	2-3
Organic phosphates	0.7-1
Total as P	5.7-8

3. Regulations and guidelines

In order to preserve the water bodies, each country has set its own effluent water quality standards. In many parts of the world including North America, Europe and Asia regulations have set effluent TP discharge limits from WWTPs at 1 mg/L (Oleszkiewicz, 2015). However, both in US and Canada, many provinces and states have also set their own phosphorous discharge limit which is usually site specific and stricter than the national guidelines. In Canada, Ontario has the most stringent TP limit due to the presence of Great Lakes. Many WWTPs in Ontario have effluent TP limits as low as 0.2 mg/L or below. According to the Great Lakes Water Quality agreement enforced on February 12, 2013, the regulatory and non-regulatory programs have set TP discharge limit of 1 mg/L for Lakes Superior, Michigan, and Huron and 0.5 mg/L for Lake Erie and Lake Ontario. Many plants in Ontario have been designed to achieve effluent TP concentrations of less than 0.4 mg/L, such as WWTPs in Lake Simcoe (<0.1 mg/L) and Kitchener (<0.4 mg/L). In USA, Great Lakes and Chesapeake Bay have the strictest TP limits of 0.5-1 mg/L and 0.3 mg/L, respectively. However, in recent years even stricter effluent TP limits have been set in the USA. For example, the Syracuse Metropolitan Wastewater Treatment Plant has a TP limit of 0.02 mg/L. Oleszkiewicz and Barnard (2006) reported many WWTPs in the US meet effluent TP concentrations between 0.03

to 0.3 mg/L with EBPR combined with tertiary P-removal technology, such as chemical trimming and filtration. The TN discharge limits are generally based on site specific and can be varied between 3 to 10 mg/L(Oleszkiewicz, 2015). Similarly, ammonia discharge limit is also assessed on a site specific basis and typical values ranged between 1 to 5 mg/L(Oleszkiewicz & Barnard, 2006; Oleszkiewicz ,2015). Refractory dissolved organic nitrogen (rDON) which are not biologically degradable are generally found in secondary clarifier effluent in the range of 1 to 2 mg/L(Metcalf and Eddy, 2014). The limits of current treatment technologies are generally set by the rDON concentration in the secondary effluent.

4.Fundamentals of biological nitrogen and phosphorus removal

4.1. Nitrogen removal

4.1.1. Conventional nitrogen removal process

Biological nitrogen removal in conventional processes takes place through the action of autotrophic and heterotrophic bacteria. This involves nitrification under aerobic conditions and denitrification under anoxic conditions.

Nitrification:

Nitrification refers to the conversion of ammonia-nitrogen into nitrate-nitrogen using biological pathway. This is performed by autotrophic microorganisms which use carbon dioxide as the source of carbon. This is a two-step biological process in which $\text{NH}_4\text{-N}$ is converted into $\text{NO}_2\text{-N}$ by ammonium oxidizing bacteria (AOB). AOBs, such as *Nitrosomonas*, *Nitrospira*, *Nitrosococcus* are more commonly found in nitrification plants. In the second step, nitrite oxidizing bacteria (NOB) bacteria convert the nitrite-nitrogen into nitrate-nitrogen through oxidation. *Nitrobacter*, *Nitrospira*, *Nitrococcus* are some of the most common NOBs in nitrifying sludge. The reactions and stoichiometry in nitrification is given below:

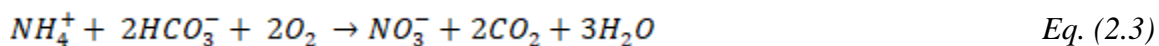
Ammonium oxidizing bacteria:



Nitrite oxidizing bacteria:



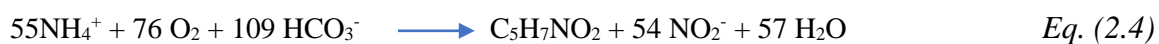
The amount of alkalinity required to carry out the nitrification can be calculated from the following equation:



From this equation, for each gram of NH₄-N to be completely oxidized into NO₃⁻ 4.57g O₂ and 7.14 g alkalinity as CaCO₃ will be required.

Nitrifying bacterial use inorganic carbon, such as CO₂ in the form of bicarbonate as the source of carbon and assimilate a portion of the NH₄-N for cellular growth and new cell synthesis. Assuming a synthesis yield of 0.15 gram VSS per gram NH₄-N oxidized by AOBs and 0.04g VSS per gram NO₂-N oxidized by NOB, the biochemical conversion of NH₄-N to NO₃-N can be represented as follows (Parker et al., 1975):

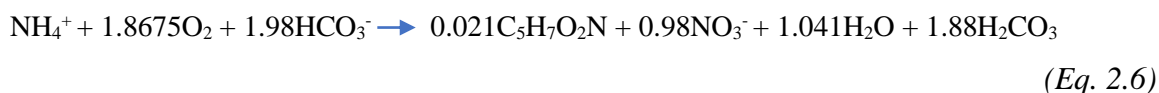
Ammonium oxidizing bacteria:



Nitrite oxidizing bacteria:



Therefore, the complete nitrification of NH₄-N with cell synthesis can be represent as follows:

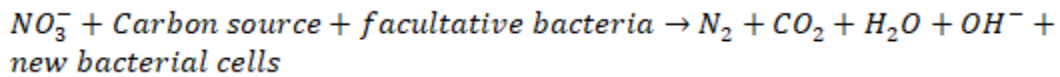


From the above equation, for each gram of NH₄-N converted to NO₃-N, 4.26g oxygen and 7.07g alkalinity (as CaCO₃) is consumed. This requirement is less than the theoretical value calculated from equation 2.3, which excluded ammonia consumption for cell synthesis.

Denitrification:

Denitrification is the process of reducing nitrate (or nitrite) into nitrogen gas by heterotrophic microorganism which uses organic substances as a source of their carbon. The denitrifiers are usually facultative, i.e., they can perform in both anoxic or oxic environment. Under oxygen limiting condition (< 0.3 mg/L) , they can strip oxygen from nitrate/nitrite to synthesize carbon compounds.

The generic equation of denitrification is given below:



(Eq. 2.7)

During denitrification, one equivalent of alkalinity is produced per equivalent of NO_3^- -N reduced, which equates to 3.57g alkalinity (as $CaCO_3$) production per gram of NO_3^- -N reduced.

The amount of biodegradable soluble COD (bsCOD) for denitrification depends on the operating condition and type of electron donor (Metcalf and Eddy, 2014). The amount of bsCOD required for denitrification can be calculated from the following equation:

$$g \text{ bsCOD} / g \text{ NO}_3\text{-N} = 2.86 / (1 - 1.42 \times Y_n)$$

where, Y_n = net biomass yield (g VSS// bsCOD)

Besides heterotrophic denitrifiers, a number of autotrophic denitrifying bacteria have also been reported in the literature. Autotrophic denitrifiers are capable of using NO_x -N as electron acceptor and a wide range of electron donors. *Paracoccus ferrooxidans* and *Paracoccus denitrificans* can oxidize zero valent iron and Fe(II) while denitrifying oxidized nitrogen (Kumaraswamy et al., 2006). These microorganisms are also found to be capable of using thiosulfate and thiocyanate as inorganic electron donor. *Thiobacillus denitrificans* and *Thiomicrospira denitrificans* are also reported to perform simultaneous sulfur and nitrogen removal by using reduced sulfur as an electron donor (Zou et al., 2016).

4.1.2 Partial nitrification and denitrification

Partial nitrification-denitrification is considered to be a more techno-economically viable process compared to the conventional nitrogen removal process (Mavinic & Turk, 1987; Van Kempen et al., 2001) Partial nitrification is the biological nitrogen removal process via nitrite where the second step of the nitrification process is restricted, thereby, accumulating nitrite which can be used as an electron acceptor for denitrification (Fig.2.4).

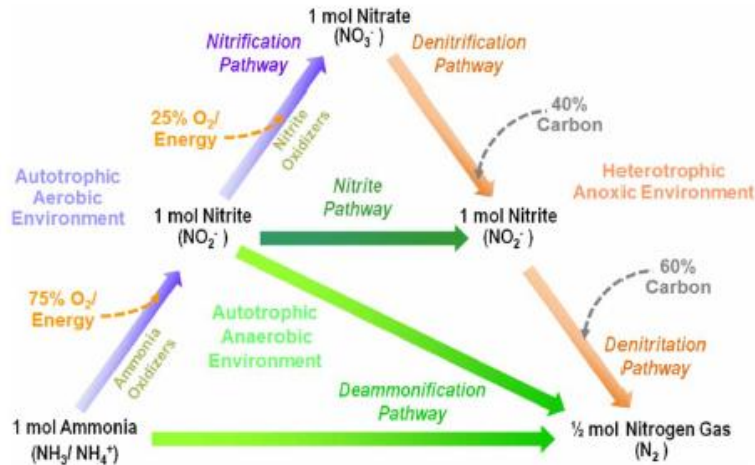
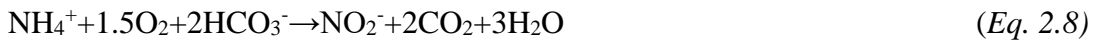


Figure 2.4 Partial nitrification-denitrification pathways (Willis et al., 2017)

This can be achieved by selectively inhibiting the growth of nitrite oxidizing bacteria (NOB) and facilitating the growth of ammonia oxidizing bacteria (AOB). Partial nitrification-denitrification is considered to be more techno-economically viable process compared to the conventional nitrogen removal process. The reactions involved in this process are shown below:

Nitrification:



Denitrification:



Overall equation for nitrification-denitrification:



Compared with conventional BNR, the main advantages of partial nitrification and denitrification via nitrite include: (a) 25% lower oxygen consumption, (b) 40% lesser carbon requirement, (c) 1.5 to 2 times faster kinetics, and (d) 40% lower sludge production (Peng & Zhu, 2006). However, nitrite toxicity and nitrous oxide production are the two most significant bottlenecks of this technology (Oleszkiewicz et al., 2015).

4.1.3. Simultaneous nitrification-denitrification (SND)

Simultaneous nitrification-denitrification is a well-established process in activated sludge systems. Many studies showed that nitrification and denitrification can occur simultaneously in a single tank activated sludge process at low DO (0.5 ± 0.1 mg/L)

conditions (Bertanza, 1997; Bueno et al., 2018; Dai et al., 2017; Helmer & Kunst, 1998). When SND proceeds via nitrite pathway, it can further reduce carbon and energy consumption for nitrogen removal (Yan et al., 2019). Most importantly, since SND can be performed in the same tank, it can significantly reduce the capital investment by reducing the number of tanks for N-removal.

SND typically occurs when the oxygen transfer within the microbial aggregates is limited. The diffusion limited oxygen transfer leads to the formation of a core-shell structure (Fig.2.5)(Sun et al., 2010). The outer aerobic shell is formed by the autotrophic nitrifying bacteria where nitrification/nitrification takes place and the inner anoxic core is formed by the denitrifying heterotrophic bacteria (DNHB), which denitrifies $\text{NO}_x\text{-N}$ into N_2 gas. The nitrification products accumulate at the core-shell interphase for generating the concentration gradient for diffusion into the inner anoxic core.

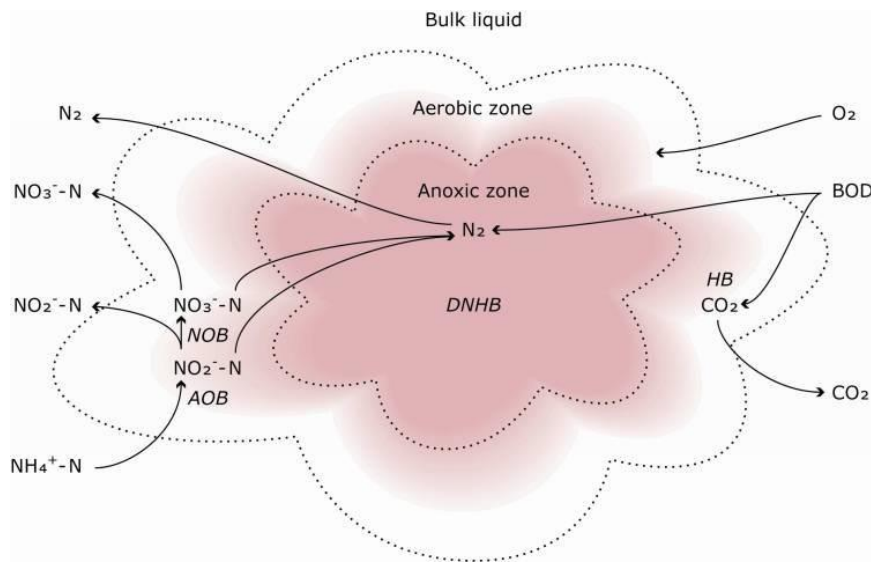


Figure 2.5 Simultaneous nitrification-denitrification in microbial aggregates (Sun et al., 2010)

SND efficiency largely depends on a number of process variables, such as C/N ratio, bulk DO concentration, characteristics of microbial aggregates, and bioreactor configuration, such as mixing condition (Jimenez et al., 2011; Yan et al., 2019). Even though the fundamental mechanism of SND is well documented, high SND efficiency is difficult to achieve in full-scale plants due to limited control over various process

variables, such as size of microbial aggregates, internal COD storage, and intra-aggregate DO profile

4.2. Enhanced biological phosphorus removal (EBPR)

Enhanced biological phosphorous removal phosphorus removal process can be achieved by subjecting the bacteria in an activated sludge process to alternating anaerobic/aerobic (or anoxic) conditions in the presence of volatile fatty acids (VFA). This condition favors the proliferation of phosphorus accumulating microorganisms (PAOs) in the activated sludge, which are able to uptake more phosphorus than they need for their normal growth. While the luxury uptake of phosphorus was initially observed by Shapiro (Shapiro et al., 1967), Bernard and co-workers established the need for cyclic anaerobic-aerobic condition for effective phosphorus removal (Barnard, 1975). The process is generally termed as enhanced biological phosphorus removal (EBPR). Although various configurations are proposed since its invention in the early 1970's, the simplest form of an EBPR is shown in Fig. 2.6.

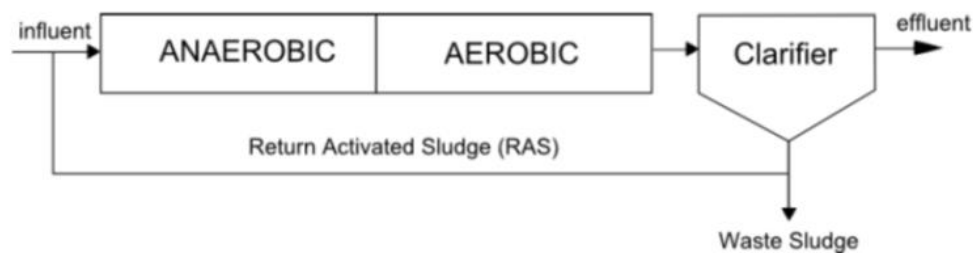


Figure 2.6 Basic configuration of an EBPR process (Janseen et al., 2002)

Under anaerobic conditions, PAOs utilize organic carbon and convert them into intracellular organic polymer commonly known as poly- β -hydroxyalkanoates (PHA) (Fig.2.7). The PHA family is comprised of three different polymers. Depending on the type of volatile fatty acid, the synthesized polymer could be composed of poly- β -hydroxybutyrate (PHB), poly- β -hydroxyvalerate (PHV), poly- β -hydroxy-2-methylvalerate (PH2MV) (Oehmen et al., 2005; Smolders et al., 1994).

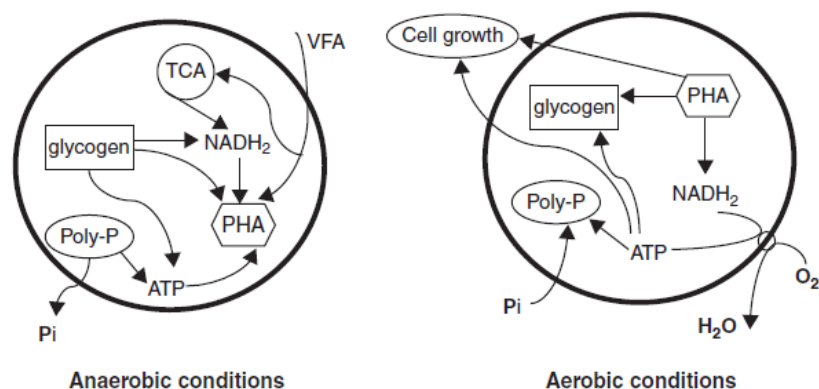


Figure 2.7 Summary of biochemical model for EBPR (Yuan et al., 2012)

The energy required for the transportation and synthesis of PHA is provided by the hydrolysis of internally stored poly-P material (Mino et al., 1998; Smolders et al., 1994). The energy required for cellular maintenance during the anaerobic stage is also provided from the hydrolysis of poly-P material. The reducing equivalent for PHA synthesis (NADH₂) comes from metabolism of glycogen (Smolders et al., 1994). The hydrolysis of polyphosphates (poly-P) followed by phosphate release results in an increase in orthophosphate concentration in the system.

During the aerobic (or anoxic) phase under carbon limited condition, stored PHA is used as a source of carbon for : (1) recovery of glycogen storage, (2) biomass growth, (3) cellular maintenance energy (Smolders et al., 1995) which results in higher uptake of orthophosphate in the aerobic (or anoxic) phase than that released in the anaerobic phase. The P-uptake by metabolic pathways in PAO is usually stored as poly-p materials within the cells. However, it has been reported that in addition to P-uptake via metabolic pathways, P can be also physically or chemically bound to biomass (Kim & Nakhla, 2009). The true biologically bound P can be distinguished from chemically/physically bound P using the perchloric acid/NaOH extraction method outlined by (De Haas et al., 2000). An increasing biologically bound P of the aerobic and anoxic sludge represents enhanced PAOs and DPAOs activity in the EBPR system, respectively (Kim & Nakhla, 2009). The overall P-removal is achieved through the routine wastage of P-enriched activated sludge.

4.2.1 Microbiology of EBPR

Identification of the dominant PAO species in the activated sludge has been a key aspect of EBPR research in the past decades. One of the dominant groups of PAOs is *Candidatus Accumulibacter Phosphatis*. This class of bacteria is akin to *Rhodocyclus* associated to subclass 2 of *Betaproteobacteria* (Hesseltmann et al., 1999). Although pure cultures of *Accumulibacter* are very difficult to achieve, their enrichment in laboratory scale EBPR reactors have been frequently observed (Lu et al. 2006; Oehmen et al., 2007). PAOs enrichment is also observed in the full-scale EBPR reactors. They typically contribute to about 5%-20% of the bacterial community in the activated sludge (Saunders et al., 2015). The *Accumulibacter* community is divided into two types (I and II) which are further divided into several clades (IA-E, IIA-G) (He et al., 2008; Peterson et al., 2008). Carvalho et al. (2007) and Oehmen et al. (2010) reported that PAO-I is capable of anoxic P-removal via nitrate and nitrite. However, PAO-II lack the nitrate reductase enzyme and capable of anoxic P-uptake via nitrite only. PAO-II commonly rely on denitrifying glycogen accumulating organisms (DGAOs) for nitrate reduction to nitrite in a complete nitrification system (Rubio-Rincón, 2017). Researchers have recently identified the complete genome sequence of Type IIA *Accumulibacter* which helped to further confirm the metabolic models previously established (Mchardy et al., 2006).

Glycogen accumulating organisms (GAO), such as *Candidatus Competibacter Phosphatis* and *Defluvicoccus* are also commonly found in EBPR systems and have been repeatedly reported for the failure of EBPR that should otherwise perform stable P-removal at the given operating conditions (Cech & Hartman, 1990; Čech et al., 1993; Lopez-Vazquez et al., 2009; Satoh et al., 1996). In contrast to PAOs, they use glycogen as a source of energy instead of poly-P; therefore, no phosphorus is released during anaerobic substrate uptake and subsequent aerobic P-removal is not observed (Čech et al., 1993). Usually, pH and temperature are considered as a selector factor for PAO/GAO in the EBPR system. Typically, substrate uptake is more favorable for GAOs at low pH (below 6.5) and less favorable at pH 7-7.5. Similarly, GAOs tend to dominate EBPR at high temperature (30°C) while PAOs dominate at moderate temperature (20°C) (Whang & Park, 2002). A subclass of both PAOs and GAOs, known as denitrifying PAOs (DPAOs) and denitrifying GAOs (DGAOs) are also found in EBPR system performing

simultaneous nitrogen and phosphorus removal (Rubio-Rincón et al., 2017). Since PAO clade II of *Candidatus Accumulibacter Phosphatis* can perform denitrification from nitrite only, presence of DGAOs can be beneficial for simultaneous nitrogen and phosphorus removal in a fully nitrifying plant.

Recent studies showed *Accumulibacter* is not the only phosphorus removing species found in EBPR. Gram positive bacteria, such as *Actinobacteria* and *Tetrasphaera* are also observed in the full-scale EBPR reactors and are able to take up orthophosphate when exposed to anaerobic/aerobic cycles (Marques et al., 2018; Nguyen et al., 2011). However, their metabolic pathways are not very well understood. Even though *Tetrasphaera* is capable of assimilating a wide range of carbon sources, such as amino acids, glutamic acid, glucose, acetate, etc., they lack the ability to synthesize polyhydroxyalkanoate as a storage polymer (Nguyen et al., 2011). Even though anaerobic substrate uptake is a key factor for the subsequent aerobic P-uptake, the presence and nature of storage polymer is largely unknown. Marques et al. (2018) reported that they are capable of denitrification using stored carbon; however, without any significant anoxic P-uptake.

4.2.2 The role of various carbon sources in EBPR performance

The availability and nature of the carbon source play a vital role on EBPR performance. Carbon directly contributes to the microbial community selection and long-term stability of EBPR process. The extent of anaerobic substrate uptake with complex carbon sources are lower than simple carbon sources (acetate and propionate) as they transformed to short chain VFAs, primarily acetate and propionate, by other microorganisms prior to uptake by PAOs. Over the past years, various types of carbon sources including natural and synthetic have been investigated in EBPR processes (Shen, & Zhou, 2016).

Acetate and propionate are the most widely studied carbon sources in EBPR research. The specific substrate utilization rate of acetate (0.20-0.26 to mg HAc/mgVSS-h) and propionate (0.23 mg HPr/mgVSS-h) by PAOs are very similar (Filipe et al., 2001; Murnleitner et al., 1997; Oehmen et al., 2005; Smolders et al., 1994). Microbial analysis showed that for acetate and/or propionate fed system, phosphorus accumulating

organisms are the dominant microbes compared to the glycogen accumulating organism (GAO) (Oehmen et al., 2005; Schuler & Jenkins, 1996) which is the primary reason for relatively stable performance of acetate and propionate fed EBPR system. However, it is also reported that excessive acetate loading can led to deterioration of EBPR performance due to proliferation of GAOs (Schuler & Jenkins, 1996). A COD/P ratio of 3 mg/mg showed higher phosphorus removal rates compared to the conventional COD/P ratio of 10 mg/mg. Under anaerobic condition, P-release to acetate uptake rate and glycogen synthesized to acetate uptake ratios were 0.43-0.73 P-mol/C-mol and 0.08-0.50 C-mol/C-mol, respectively (Table 2.2). Gly/C ratio of 0.5 or lower indicates the favorability of PAOs over GAOs in the EBPR systems (Shen, N. & Zhou, 2016). For acetate-fed systems, metabolic models were combined with ASM models to obtain stoichiometric and kinetic parameters of EBPR which are summarized in Table 2.3. It can be seen from Table 2.3, that at low temperature, the stoichiometry of EBPR is less affected compared to the kinetics.

Table 2.2 Carbon Transformation in EBPR from various sources (Shen & Zhou, 2016)

Carbon source	anaerobic phase			aerobic phase
	P/C (P-mol/C-mol)	PHA/C (C-mol/C-mol)	Gly/C (C-mol/C-mol)	P-uptake rate (mmol/g VSS.h)
Acetate	0.45-0.73	0.62-1.48	0.08-0.50	0.23-0.48
Propionate	0.23-0.44	0.52-1.39	0.05-0.49	0.41-0.72
Glucose	0.0059-0.121	0.36-0.44	(-0.28) – (-0.12) ^a	-
Ethanol	0.1-0.4	1.0-1.2	0.63-0.8	0.03-0.23
Glycerol	0.2	0.31	0.25	-
Glutamate	0.73	0.61	0.49-0.64	1.14-1.17 ^b
Aspartate	0.55	0.95	0.55-0.57	1.14-1.17 ^b
Acetate model	0.5	1.33	0.50	-
Propionate model	0.42	1.22	0.33	-

^a indicates formation not degradation

^b P-uptake/P-release ratio

P/C- phosphorus release to carbon uptake

PHA/C- PHA synthesized to carbon uptake

Gly/C- glycogen synthesized to carbon uptake

Table 2.3 Stoichiometric and kinetic parameters of EBPR at various temperatures in acetate fed systems (Henze et al., 1999; Liao et al., 2015)

Parameter	Description	Unit	20°C	28°C	32°C
q_{PHA}	Acetate uptake rate constant	gCOD/gCOD.day	3.0	17.8	8.2
q_{PP}	Polyphosphate storage rate constant	gPO ₄ -P/gCOD.day	1.5	3.6	2.9
μ_{PAO}	Maximum growth rate of PAOs	1/day	1	4.7	2.5
K_{PHA}	Saturation coefficient for PHA in PAOs	gCOD/gCOD	0.010	0.014	0.015
Y_{PO_4-P}	Yield of Poly-P required per PHA storage	g PO ₄ -P/g-COD	0.40	0.23	0.10
Y_H	Yield of heterotrophic organism growth	gCOD/gCOD	0.625	0.821	0.670
Y_{PAO}	Yield of PAOs growth	gCOD/gCOD	0.625	0.821	0.670

Propionate is another popular carbon source for EBPR. In fact, it is more suitable for EBPR as less energy and lower poly-P is consumed for propionate uptake compared to acetate uptake (Table 2.2). It has been reported in the literature that during acclimatization, *Accumulibacter* increased from 4.54% to 9.53% and 4.38% to 41.5% of the total biomass in acetate- and propionate-fed systems, respectively (Lv et al., 2014). On the other hand, GAOs varied from 1.18% to 2.22%. It has been reported that Gly/C ratio was lower in propionate feed system compared to the acetate feed system (0.32 C-mol/C-mol versus 0.69 C-mol/C-mol) which further supports that propionate favours PAOs over GAOs (Carvalho et al., 2007).

Butyrate was also investigated as a carbon source for EBPR due to its structural similarity to 3 hydroxybutyrate, the monomer for polyhydroxybutyrate (PHB) (Lemos et al., 1998; Pijuan et al., 2009; Zaman et al., 2019). However, butyrate was not found as effective as acetate or propionate both from stoichiometric and kinetic aspects. It has been reported in the literature that butyrate uptake rate (0.017 mmol C/gVSS-min) by PAOs is 70% slower than acetate (0.058 mmol C/gVSS-min) and propionate (0.051 mmol C/gVSS-min) (Pijuan et al., 2009). Carbon recovery ratio (PHA produced/substrate uptake) with butyrate was also found to be significantly lower than acetate and propionate. The yield of polymer (Y_p/s , mg polymer/mgCOD) produced per carbon consumed was found to decrease from acetate (0.97) to propionate (0.61) to butyrate (0.21).

Compared to acetate and propionate, glucose was found to be a poor carbon substrate for EBPR due to lack of carbon utilization and relatively higher abundance of GAOs in the microbial community (Cech & Hartman, 1990). A lower P-release and carbon transformation was observed in the glucose fed system. The P-release/C-uptake and PHA/C-uptake ratio was found to be 0.0059-0.121 mol-P/mol-C and 0.36-0.44 mol-C/mol-C, respectively (Pijuan et al., 2009). Ethanol, aspartate, and glutamate were also investigated as carbon sources for EBPR (Pijuan et al., 2009). Aspartate and glutamate yielded good EBPR performance with a P-uptake/P-release ratio of 1.14-1.17. The phosphorus and carbon transformation ratios of these compounds were also found to be comparable to acetate and propionate (Table 2.2).

Other than synthetic carbon sources, organic waste materials, such as crude glycerol, food waste, primary sludge, and waste activated sludge can also be used as a low cost carbon source in EBPR. However, very limited studies have been documented in the literature using waste byproducts as C-source in EBPR. Very little is known regarding the stoichiometry, kinetics, and metabolism of complex carbon sources from such waste byproducts in EBPR process.

Crude glycerol (CG), a byproduct of biodiesel industry, was found to be effective for enhancing EBPR performance (Coats et al., 2015; Guerrero et al., 2015). Good EBPR stability was found with CG as a carbon supplement even with a small fraction of PAOs. A novel control strategy through addition of CG using a feedback as well as feed forward control strategy to maintain the effluent TP concentration around 1 mg/L (Guerrero et al., 2015). However, the stoichiometric and kinetic aspects were not studied in detail in these studies. Several full-scale demonstration studies were conducted with glycerol as a carbon source in EBPR (Andalib & Ledwell, 2016; Andalib et al., 2015; Andalib et al., 2017). It was found that glycerol can be utilized in EBPR in two different pathways: (1) fermentation to acetate and propionate and subsequent uptake of these VFAs by PAOs/DPAOs to synthesize PHA, (2) direct internalization of glycerol in the form of organic polymer and ultimate conversion to PHA by bacteria that are not commonly classified as PAOs/DPAOs in EBPR literature. This explains previous studies where successful EBPR performance was observed with very low fraction of PAOs (Coats et al., 2015).

Disposal of waste sludge is of great concern in many wastewater treatment plants. Other than glycerol fermentation, VFAs can be also generated from waste organics, such as waste activated sludge (WAS) and primary sludge (PS). Sludge fermentation usually produces short chain VFAs with two to five carbon length (Moser-Engeler et al., 1998). Tong and Chen (2007) reported that the P-removal efficiency was higher with fermented WAS liquor compared to the acetate fed system (98.7% versus 71.1%). The ratio of sp. P-uptake rate to sp. P-release rate was also higher compared to the acetate-fed system (0.13 for fermented WAS versus 0.09 for acetate). Similar results with fermented WAS for simultaneous N and P removal (Ji & Chen, 2010) were observed; better nitrogen removal efficiency (99% versus 79%) was observed with fermented sludge vs what due to higher activity of DPAOs and nitrite reductase enzyme. Besides waste sludge, organic rich wastewaters, such as pulp & paper wastewater, agri-food wastewater were found to be useful for enhancing EBPR performance. It has been reported that agri-food wastewater, such as tomato processing and milk bottling wastewater had PAO activity comparable to acetate fed system (Fernandez et al., 2011).

Alternative to biological processes, chemical treatment was also found to be an effective process for carbon recovery both from primary and secondary sludge. Park et al. (2011) investigated the potential use of secondary sludge ozonolysate as a carbon source for EBPR. It was found that a significant fraction of COD in the ozonolysate (36% of the COD) was biodegradable and the P-removal efficiency was about half that of acetate while N-removal efficiency was comparable to acetate. Kim et al. (2009) reported the solubilization of secondary sludge using H₂O₂ treatment. The solubility (sCOD/TCOD) increased with increasing peroxide dosage. At a dosage of 1.6M H₂O₂, total solids reduction of 35% and solubility of approximately 50% was achieved.

4.2.3 Commercial technologies for carbon recovery from municipal biosolids for BNR optimization

With the increased focus on the carbon footprint of wastewater treatment and the rapidly emerging paradigm shift towards resource recovery, the utilization of indigenous organics as a renewable resource has elicited significant interest recently. Several full-

scale processes have been implemented for producing value added products, such as fertilizers, carbon for BNR, and biofuel (via incineration).

4.2.3.1 Kemwater Recycling Process

Krepro (Kemwater Recycling Process) is a commercial process for resource recovery in municipal wastewater treatment plant. It is a thermo-chemical sludge pretreatment process that offers the potential for resource recovery from WAS via following product lines: (1) biofuel (based on incineration), (2) phosphate fertilizer, (3) carbon recovery for biological nutrient removal. The process was operated for more than 3 years at Helsingborg WWTP, Sweden (Hansen et al., 2000, Levlin et al., 2002; Ødegaard et al., 2002). Fig. 2.8 shows the process flow diagram for the Krepro process.

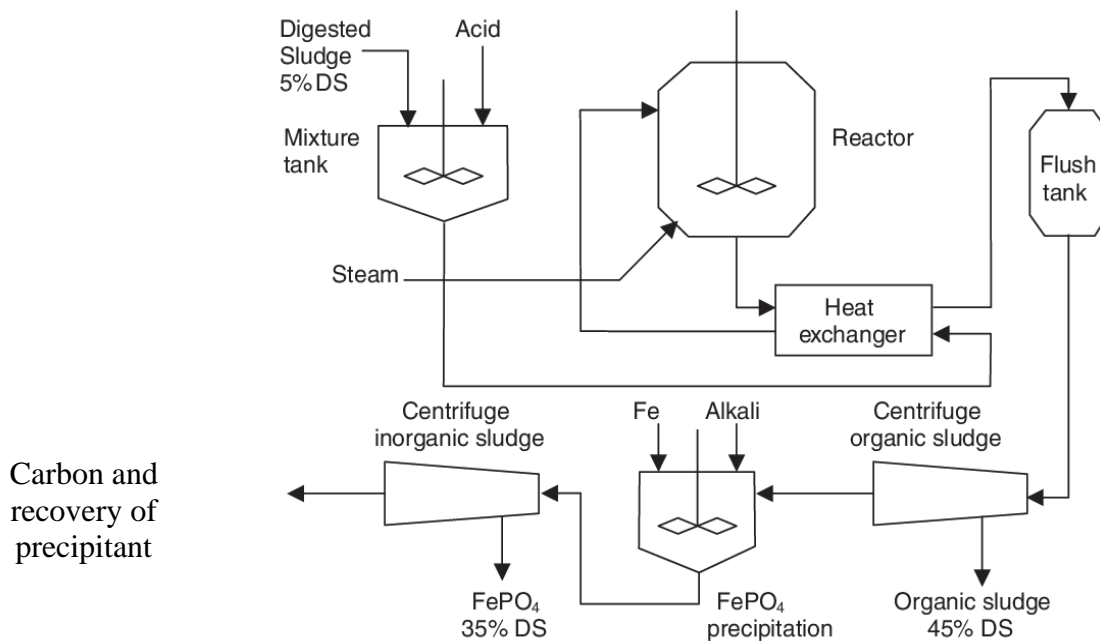


Figure 2.8 Process flow diagram for Krepro process (Ødegaard et al., 2002)

The influent sludge is thickened (5%-7% DS) prior to acidification by H_2SO_4 to a pH between 1 and 2. The acidified sludge is heated at 140°C for 30-40 min in a pressured vessel (3-4 bar) followed by rapid depressurization, which results cell lysis and solubilization of organics to a great extent (~33% for raw sludge and ~25% for digested sludge). The recovered carbon was found to be effective for denitrification where

performance was comparable to acetate. Table 2.4 shows the characteristics of recovered carbon source from digested and undigested sludge.

Table 2.4 Characteristics of the carbon source based on digested (6% DS) and raw (4% DS)

Parameter	Unit	Undigested Sludge	Digested Sludge
sCOD	mg/l	13000	11000
VFA	mg/l	1100	1800
SN	mg/l	1600	820
COD/N	g/g	8.1	13.4
rbCOD	%	5.5	12.7
Denitrification rate (acetate)	mgNO _x -N/ gVSS.hr	1.9-2.2 (2.8)	1.9-3.4 (3.8)

4.3.2.2. Athos™ hydrothermal oxidation process

Hydrothermal oxidation (HTO) processes are typically operated at high temperature (150-350°C) and pressure (40-200bar). HTO has the potential to utilize sludge as a source of renewable energy and materials. Athos™ is a commercial HTO process (marketed by Veolia Water Technologies) operated at moderate temperature (245°C) and pressure (45bar) and uses oxygen for solubilization of organic matter (Fig. 2.9) (*Athos-Hydrothermal Oxidation*, n.d.). The contact time typically varies between 30-60 min. This process converts sludge into water, carbon dioxide, mineral based solids, and readily biodegradable carbon (mostly VFA). The process can achieve 75% COD abatement, and a very high degree of mineralization (less than 5% organic in the solid residue). The organic nitrogen is converted to ammonia and approximately 10%-20% of the total nitrogen was removed in the off gas which is catalytically oxidized before release to the atmosphere (Luck et al., 1998). The treatment of the ammonia-rich supernatant liquor recovered by the standard wet oxidation processes is improved to reach about 70 % removal of nitrogen, allowing the recycling of this easily biodegradable liquor with the plant influent, as a cheaper carbon-containing source for biological denitrification /dephosphatation. (Rose et al., 2000). The waste heat is fully recoverable

via preheating the thickened sludge, resulting in no external heat requirement. This process also does not require additional dewatering as the oxidized sludge is dried to approximately 50%DS. The readily biodegradable carbon (equivalent to 15% of the total COD in sludge) can be utilized for BNR augmentation and the highly mineralized inert solid residue can be utilized in ceramic industry.

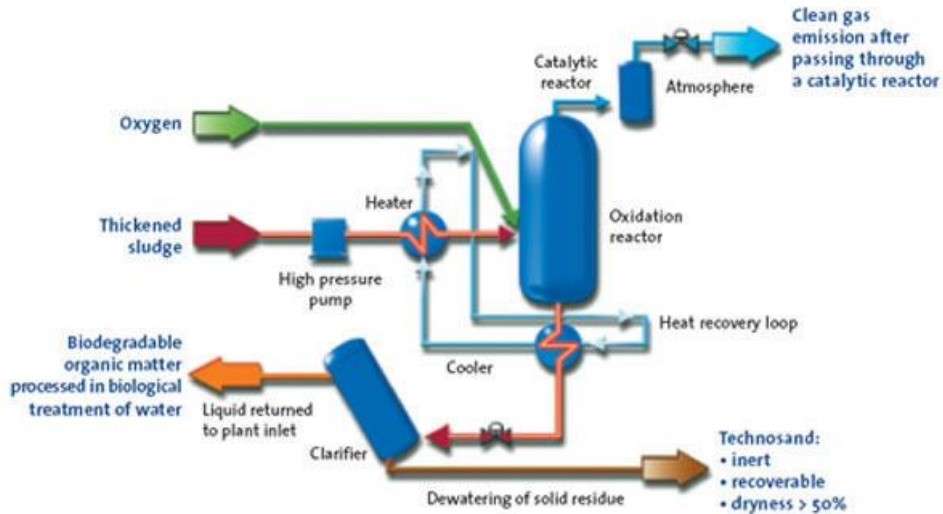


Figure 2.9 Schematic of Athos™ process (*Athos-Hydrothermal Oxidation*, n.d.)

4.3.2.3. Lystek THP® hydrolysis process

Lystek THP® is a thermal-mechanical-alkaline hydrolysis process which involves high speed shearing, alkaline treatment, and low-pressure steam treatment in a single compact system. The process is an outcome of the research at The University of Waterloo and was commercialized by Lystek International Inc. in 2000. Lystek THP® has been implemented in 8 full-scale plants in Canada and 3 full-scale plants in USA. The hydrolysis is typically conducted at 70-75°C, pH 9.5-10, and pressure 1 atmosphere and it converts biosolids into value added products which can be used across three different product line: (1) LysteGro®, class A biosolids fertilizer, (2) LysteMize®, digester enhancement, and (3) LysteCurb®, BNR augmentation. The Lystek treated sludge typically contains 40%-50% of COD as soluble COD (sCOD) and an order of magnitude higher VFAs (10-15 g/L) compared to traditional biosolid treatment processes. Table 2.5 shows typical characteristics of Lystek sludge.

Table 2.5 Typical characteristic of Lystek product (“Lystek International Inc.” ; Zaman et al., 2019)

Parameter	Unit	Lystek Product
COD	mg/l	105,000-150,000
sCOD	mg/l	50,000-60,000
VFAs	mg/l	10,000-15,000
TN	mg/l	8000
SN	mg/l	3,600
TP	mg/l	3,500
SP	mg/l	300
COD/N	g/g	19
COD/P	g/g	43
Viscosity	cP	4,000-6,000
E.coli	CFU/g	<10 (not detectable)
Fecal coliforms	MPN/g	<1.8 (not detectable)

A schematic for resource recovery via Lystek THP® process is shown in Fig.2.10. LysteGro® is enriched with both macro (N,P,K)- micro (Ca, S, Fe, Mg) nutrients and approximately 5% organics. A field trial for corn production with LysteGro® showed an average increase of 16.5 bushels/acre compared to commercial fertilizers (Brown, 2017). For digester enhancement, recycling about 25% of the treated sludge to the digestors increases biogas production by 13% (yield increased by 40% or more) and decreases solid disposal by 20%-30%.

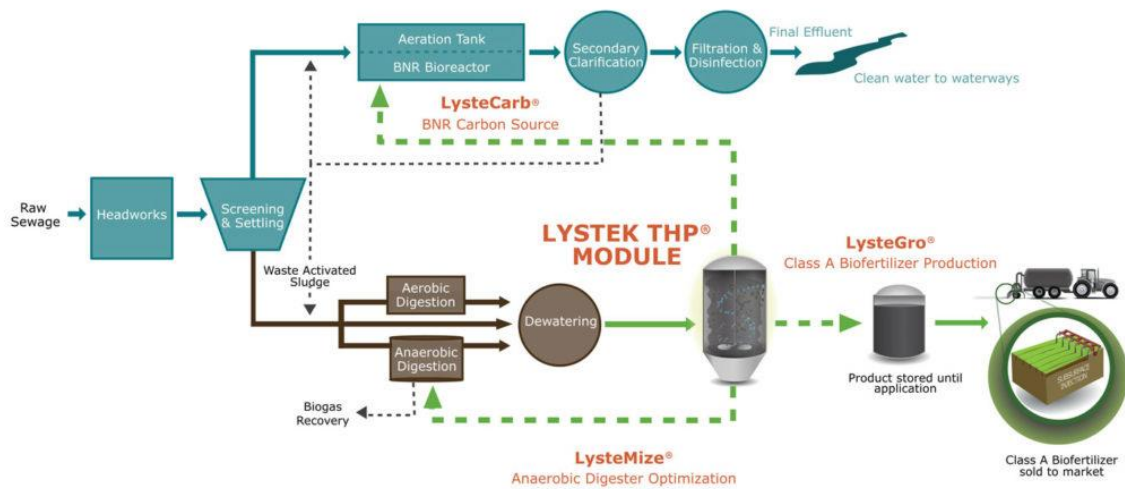


Figure 2.10 Resource recovery in WWTP via Lystek THP® sludge treatment process (“Lystek International Inc.”)

LysteCurb® was implemented as an alternative carbon source for BNR in bench scale trials and both nitrogen and phosphorus removal was found to be enhanced with a nitrogen and phosphorus removal efficiencies of about 67% and 98%, respectively (Singh et al., 2016; Zaman et al., 2019). Denitrification rate was superior to ethanol and about 40%-50% that of acetate. Phosphorus removal rate was 30%-40% to that of acetate.

4.3. Denitrifying EBPR (DPR)

In conventional EBPR, an anaerobic/aerobic sequence is generally used where PAOs use O_2 as the terminal electron acceptor in the electron transport chain. However, EBPR is commonly integrated with nitrogen removal in BNR process where nitrates and nitrites are common intermediates of nitrification and denitrification process. While nitrites are inhibitory to PAOs, they can be used as an electron acceptor by denitrifying PAOs (Kuba et al., 1996). Denitrifying phosphorus accumulating organisms (DPAOs) are more resistant to nitrites inhibition than PAOs. Table 2.6 shows that the inhibition concentrations widely varied in the literature and it would be more appropriate to report inhibition concentration normalized to biomass concentration in the reactor.

Table 2.6 Response of PAOs to nitrite in aerobic and anoxic EBPR

Reactor configuration	Inhibition level (NO ₂ -N, mg/L)		NO _x Source	References
	Aerobic uptake	Anoxic uptake		
An/O	10-15	5-8	Synthetic	(Meinhold et al., 1999)
An/Ax/O	6	12	Synthetic	(Saito et al., 2004)
An/O	-	>40	non-synthetic	(Ahn et al., 2001)
An/O and An/Ax	1(A/O);	3(A/A)	Synthetic	(Yoshida et al., 2006)
An/O/Ax/O	5	>20	Synthetic	(Sin et al., 2008)
An/O/Ax/O	-	>10	Synthetic	(Lee et al., 2001)
An/Ax	-	>35	Synthetic	(Hu et al., 2003)

Saito et al. (2004) reported that while at 7 mg NO₂-N/L concentration, aerobic P-uptake was completely inhibited. more than 90% anoxic P-uptake activity was retained. This facilitates simultaneous N & P removal by DPAOs at a relatively lower energy and carbon consumption. In addition, denitrifying phosphorus removal via nitrite pathway can further reduce the cost of aeration energy and external carbon source by 25% and 40%, respectively (Peng & Zhu, 2006). It has been well documented that phosphorus removal can be achieved in the presence of nitrate in activated sludge system (Barker & Dold, 1996; Chung et al., 2006; Kern-Jespersen & Henze, 1993; Kern et al., 1994; Tsuneda et al., 2006; Wang et al., 2015; Zou et al., 2006). However, due to the lower energy production from nitrite/nitrate compared to oxygen, a lower phosphorus uptake rate was reported, with the maximum sp. Phosphorus uptake rate of anoxic (NO₃), anoxic (NO₂), and aerobic EBPR of 27.7, 23.6, 44.85 mg PO₄-P/g VSS.h, respectively. Furthermore, greater PHA consumption for P-uptake was observed when nitrate was used as an electron acceptor (20.1 mg/gVSS for aerobic versus 27.1 mg/g VSS for anoxic). The ratio of mg P-removed/mg PHA-consumed was found to be 0.68 (O₂) and 1.09 (NO₃). Therefore, from both stoichiometric and kinetic points of view, anoxic phosphorus uptake was found to be less efficient compared to the aerobic phosphorus uptake. However, reduced energy and carbon consumption for simultaneous N & P removal make DPR economically attractive over conventional EBPR. It was also reported that switching the electron acceptor from nitrate to nitrite, the P-uptake rate did not decrease significantly (less than 15% reduction) (Hu et al., 2003); however, nitrite can save a significant

amount of carbon and energy compared to nitrate as previously mentioned. Another advantage of nitrite over nitrate is that nitrite can selectively washout GAOs which is a key bottleneck of EBPR technology. GAOs were selectively washed out from a propionate-fed EBPR system by providing nitrite as an electron acceptor (Tayà et al., 2013). The SBR was spiked with 2 dosages of 20 mg NO₂-N/L, each, during the anoxic cycle. The response of various microorganisms involved in the EBPR with respect to different electron acceptors and donors is shown in Table 2.7.

Table 2.7 Comparison of the performance of various PAO/GAO subgroups with different electron donor/acceptor combinations (Oehmen et al., 2010)

	Preferred VFA	Denitrification Capacity	
		NO ₃ ⁻	NO ₂ ⁻
<i>Accumulibacter</i> PAO I	Acetate & Propionate	√	√
<i>Accumulibacter</i> PAO II		×	√
<i>Competibacter</i> GAO	Acetate		
Sub-group 1, 4, 3		√	×
Sub-group 3, 7		×	×
Sub-group 6		√	√
<i>Defluviicoccus</i> DF GAO I	Propionate	√	×
<i>Defluviicoccus</i> DF GAO II		×	×

It is clear from Table 2.7 that with propionate as the carbon source, and nitrite as sole electron acceptor, none of the GAO species was able to survive in the EBPR system. Additionally, DPR via nitrite can save 22.3% of PHA for phosphorus removal and 49.4% of PHA for nitrogen removal (Peng et al., 2011). This makes the EBPR via nitrite pathway advantageous to nitrate pathway. However, limited studies have been reported in the literature on denitrifying phosphorus removal via nitrite pathway (Frison et al., 2016; Peng et al., 2011; Zeng et al., 2011; Zeng et al., 2013; Zhang et al., 2010).

In most of the EBPR literature, nitrite was considered more like an inhibitor rather than an electron acceptor for DPAOs either in aerobic or nitrate based DPAO systems (Bortone et al., 1996; Kuba et al., 1996; Meinhold et al., 1999; Saito et al., 2004; Zhou et al., 2008). The threshold concentration for NO₂-N inhibition was reported to be in the range of 8-10 mg NO₂-N/L. However, this range is highly dependent on sludge type and acclimatization process. For example, Hu et al. (2003) acclimatized the sludge in

anaerobic/anoxic reactor with an initial $\text{NO}_2\text{-N}$ concentration of 40 mg/L for a year and half, and batch studies with various nitrite concentrations showed nitrite is not inhibitory to EBPR upto 35 mg $\text{NO}_2\text{-N/L}$. Table 2.7 shows the inhibition level of nitrite in aerobic and anoxic EBPR. The threshold nitrite concentration could vary depending on the relative percentage of DPAOs (nitrite) to other types of microorganisms.

While denitrifying EBPR using nitrate can be achieved by integrating complete nitrification and denitrification (Kuba et al., 1996), denitrifying EBPR via nitrite can be achieved by combining short-cut nitrification with EBPR (Guisasola et al., 2009).

Recently, integrated nitrification-denitrification and phosphorus removal has elicited significant attention and considered as a feasible alternative to traditional EBPR process. Integrating SND with EBPR shows a viable pathway for simultaneous nitrogen and phosphorus removal. Numerous studies have been reported in the literature on SNDPR for nutrient removal from wastewater. The key advantage of SNDPR include: (1) less sludge production, (2) reduced aeration demand, and (3) lower COD requirement for combined N and P removal. The microbial consortium of SNDPR primarily consist of ordinary heterotrophs (OHO), nitrifiers, phosphorus accumulating organisms (PAOs), and glycogen accumulating organisms (GAOs). A subgroup of PAOs, commonly known as denitrifying PAOs (DPAOs) plays a significant role on N and P removal as they can utilize both nitrate and nitrite as electron acceptors. The role of DPAOs on denitrifying phosphorus removal has been well documented in the literature). Table 2.8 summarizes the nutrient removal performance of various SNDPR systems reported in the literature.

Table 2.8 Operating conditions and nutrient removal performance in SNDPR systems

Process	Wastewater	C/N	SRT	DO	SND,%	Removal Efficiency		References
						TN	TP	
An-Oxic	Municipal WW	7	5	0.4	-	92	87	(Jimenez et al., 2014)
An-IA ^a	Municipal WW	8	11	1.0	-	83	81	(Roots et al., 2020)
An-O-Ax	Synthetic WW	10	15	-	-	83	92	(Tsuneda et al., 2006)
Floc-SNDPR	Septic tank WW	7	11	1.0	49	78	94	(Wang et al., 2015)
Granule-SNDPR	Domestic WW	11	20	2-3	68	52	71	(Wang et al., 2009)
Fixed-bed SNDPR	Synthetic WW	7	-	3-4	31	70	90	(Rahimi et al., 2011)
Floc SNDPR	Synthetic WW	16	25	1.0	79	90	97	(Li et al., 2019)
Floc-SNDPR	Domestic WW	7	-	2.0	-	75	93	(Coma et al., 2012)
Granule-SNDPR	Synthetic WW	7	30	1.8	75	80	90	(Bassin et al., 2012)
Granule-SNDPR	Synthetic WW	10	-	5.0	74	94	98	(He et al., 2016)
Biofilm-SNDPR	Synthetic WW	13	15	4.0	75	82	84	(Yang et al., 2010)
Ax-O	Municipal WW	5 ^b	5	1.4	50	90	76	(Yang et al., 2016)

a. IA- Intermittently aerated

b. sCOD/N

It can be seen that the majority of the SNDPR studies reported in the literature operate at moderate to high DO (1 mg/L and above) and high COD/N ratio (7 to 15). These high COD/N ratios are difficult to attain in municipal wastewater without carbon supplementation. However, carbon supplementation is an additional cost for wastewater treatment plants and compromises the true benefit of SNDPR. Moderate to high DO concentration is usually found to be unfavorable for the enrichment of DPAOs due to a lack of expression of nitrite reductase enzyme in *Accumulibacter* PAOs. Microbial communities at moderate to high DO were found to be dominated by *Candidatus Accumulibacter* PAOs (50%-70%), *Competibacter* and *propionivibrio* GAOs (15%-25%), and *Candidatus Accumulibacter* DPAOs (15%-25%)(Roots et al., 2020; X. Wang et al., 2015). Therefore, high DO anaerobic-aerobic operations are more favorable for combined SND and aerobic P-removal than simultaneous nitrification-denitrifying P-removal (Roots et al., 2020; Q. Yang et al., 2016). Also, the presence of GAOs usually requires a higher COD/N ratio for denitrifying P-removal, as evidenced in the literature

(Bassin et al., 2011; He et al., 2016; Li et al., 2019). The aforementioned studies reported that at DO concentrations of 1-4 mg/L, and COD/N ratios of 7-10, P-removal via DPAOs accounted for only 30%-50% of the total P-removed. The lower anoxic P-removal was primarily due to the competition from GAOs and high DO operation.

5. Synopsis of the literature

Over the past four decades, BNR has been considered as an effective approach for limiting nutrient discharges to the waterways. Carbon source and aeration, being the two most important aspects of BNR technology, were the focus of research over the past years. Synthetic carbon sources were primarily investigated, and acetic acid, propionic acid, and methanol were found to be the most suitable form of carbon for BNR. However, their non-renewable nature and high cost limit the application in full-scale wastewater treatment plants. Therefore, research for alternative carbon source is inevitable. Waste organic products, such as crude glycerol, agro-food wastewater, and WAS fermentation liquor were investigated as alternative carbon to synthetic carbon sources and were found to be comparable to synthetic carbon sources.

Aeration is the most energy intensive operation in wastewater treatment plant and accounts for 45%-75% of plant energy cost (Gu et al., 2017). Among the emerging technologies for nutrient removal, simultaneous nitrification-denitrification and phosphorus removal (SNDPR) attracted significant attention in recent years because of its lower aeration requirement, efficient carbon utilization, simultaneous N & P removal, and lower sludge production. The majority of the reported studies were limited to two sludge A₂N process. Even though single sludge SNDPR has simplified operations and maintenance, only a limited number of studies have been reported in the literature for SNDPR in single sludge system. Moreover, majority of these studies were conducted with either synthetic wastewater with high COD (VFA)/N ratio (10 or more) or VFA enriched septic tank wastewater which is not representative of the real municipal wastewater composition. Anoxic P-uptake in these studies were limited to 30%-50% of the total P-removed which represents lack of DPAOs enrichment in the mixed liquor.

6. Knowledge gaps

To date, acetate and propionate were primarily utilized as supplemental carbon sources in EBPR research for both synthetic and real wastewater. Most of the metabolic studies on EBPR were done on acetate as carbon source. The metabolic pathways for more complex carbon sources are still largely unknown. While the concept of carbon recovery via primary sludge (PS) and waste activated sludge (WAS) fermentation is already practiced (Moser-Engeler et al., 1998; Thomas et al., 2000; Tong and Chen, 2007; Ji and Chen, 2010; Ji et al., 2010), studies reporting the capacity to recover carbon from digested sludge is scant. While the extent of VFA production will be higher for undigested sludge, the carbon recovery from digested sludge would enable WWTP to simultaneously improve energy and carbon recovery. Hydrothermal treatment was found to be an effective method for solubilization of digested sludge readily biodegradable carbon (Haraguchi et al., 2006; Shanableh, 2000). However, this process is typically operated at high temperature and pressure which result in higher operational cost and capital investment. Recently, a relatively low temperature thermal-alkaline hydrolysis process (Lystek®) has been reported for solubilization of readily biodegradable carbon of raw as well as digested sludge (Singh et al., 2016). The Lystek product was reported to have 40%-50% of COD as soluble COD (sCOD) and an order of magnitude higher VFAs (10-15 g/L) compared to traditional biosolid treatment processes. However, to the best of our knowledge no study investigated the effectiveness of Lystek product as a carbon source in EBPR.

In recent years, even though SNDPR evolved as a promising alternative to traditional BNR, the majority of the SNDPR studies reported in the literature operated at moderate to high DO (1 mg/L and above) and with high COD/N ratio (7 to 15) synthetic wastewater. These high COD/N ratios are difficult to maintain in municipal wastewater without carbon supplementation. However, carbon supplementation is an additional cost for wastewater treatment plants and forfeit the true benefit of SNDPR. Besides, SNDPR from real municipal wastewater without VFA supplementation is limited to two full-scale studies (Jimenez & Dold, 2014; Yang et al., 2016) and one bench scale study only (Roots et al., 2020). All three aforementioned studies operated at a DO concentration of 1-2 mg/L and observed limited DPAOs enrichment in the mixed liquor. Anoxic P-uptake

was reported upto a maximum of 30% of the total P-removed (Roots et al., 2020; Wang et al., 2015; Yang et al., 2016) Moreover, the nutrient removal mechanism was primarily claimed to be SND via ordinary heterotrophs and mostly aerobic P-uptake via PAOs. Therefore, SNDPR with real municipal wastewater (without VFA supplementation) with improved anoxic P-uptake is yet to be explored.

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Chapter 3

Enhanced Biological Phosphorus Removal Using Thermal Alkaline Hydrolyzed Municipal Wastewater Biosolids

1.Introduction

Nutrients in wastewater effluents, i.e. nitrogen (N) and phosphorus (P) have elicited significant interest because of eutrophication of lakes in different parts of the world. According to the USEPA, nearly 25% of the water body impairments are caused by nutrient-related issues (USEPA, 2007). In order to minimize the extent of nutrient impairments from point sources, stricter jurisdictional regulations for N & P discharges have been imposed.

Enhanced biological phosphorus removal (EBPR) is a sustainable and environmentally friendly engineered wastewater treatment process that is capable of maintaining a low effluent phosphorus concentration and should be considered as the first line of defense for phosphorus reduction. The fundamental principle of EBPR is the alternating anaerobic-aerobic condition that promotes the growth of phosphorus accumulating organisms (PAOs) which can store VFAs under anaerobic conditions and utilize them under aerobic conditions along with phosphorus uptake (Adrian Oehmen et al., 2007). However, EBPR is commonly supplemented with external carbon due to lack of volatile fatty acids in most municipal wastewater treatment plant's influent (Fernandez et al., 2011; Park et al., 2011; Coats et al., 2015). Synthetic carbon sources were primarily investigated and acetic acid and propionic acid were found to be the most suitable forms of carbon for EBPR (Schuler and Jenkins, 1996; Carvalho et al., 2007; Lv et al., 2014; Shen and Zhou, 2016). Biochemical modeling of EBPR is primarily focused on acetate and propionate as the carbon source (Hesselmann et al., 2000; Oehmen et al., 2005; Yagci et al., 2003). Puig et al. (2007) reported that ethanol can also be used as a carbon source in EBPR and its performance is comparable to propionate. Zengin et al. (2011) investigated the impact of aspartate and glutamate on the performance of EBPR and found stable EBPR operation with microbial community comparable to full-scale EBPR plants. However, their non-renewable nature and high cost limit full-scale application.

Crude glycerol (CG), a byproduct of biodiesel industry, was found to be effective for enhancing EBPR performance (Coats et al., 2015; Guerrero et al., 2015). Good EBPR stability was found with CG as a carbon supplement even with a small fraction of PAOs. A novel feed forward control strategy was developed through addition of CG as a carbon source to maintain the effluent TP concentration around 1 mg/L (Guerrero et al., 2015).

Agro-food wastewater, such as tomato processing and milk bottling wastewater also showed EBPR performance comparable to acetate fed system (Fernandez et al., 2011).

With the increased focus on the carbon footprint of wastewater treatment and the rapidly emerging paradigm shift towards resource recovery, the utilization of indigenous organics for biological nutrient removal in general and EBPR in particular is eliciting interest. The concept of carbon recovery via primary sludge (PS) and waste activated sludge (WAS) fermentation is already practiced (Moser-Engeler et al., 1998; Thomas et al., 2000; Tong and Chen, 2007; Ji and Chen, 2010; Ji et al., 2010).

Sludge fermentation usually produces VFAs with two to five carbon length (Moser-Engeler et al., 1998). Short chain VFAs produced from the alkaline fermentation of PS and WAS were investigated as the potential carbon source for EBPR. It was reported that the P-removal efficiency was higher with fermented WAS liquor compared to the acetate fed system (98.7% versus 71.1%). The ratio of specific P-uptake rate to specific P-release rate was also higher compared to the acetate-fed system (0.13 for fermented WAS versus 0.09 for acetate). Similar observations were made when optimizing the operation of a full-scale EBPR plant with primary sludge fermentation (Thomas et al., 2000). It was hypothesized that glycogen-accumulating organisms (GAOs) might have a competitive advantage over PAOs in acetate-fed system. While PAOs have a competitive advantage over GAOs for higher order VFAs, such as propionate and butyrate which are commonly found in fermented sludge liquor (Thomas et al., 2000). Ji and Chen (2010) reported simultaneous N and P removal via the denitrifying phosphorus removal pathway using alkaline fermented WAS as carbon source. Enhanced nitrogen removal efficiency (99% versus 79%) was observed with fermented sludge due to higher activity of DPAOs and nitrite reductase enzyme.

Alternative to biological processes, chemical treatment was also found to be an effective process for carbon recovery both from primary and secondary sludge. Park et al. (2011) investigated the potential use of secondary sludge ozonolysate as a carbon source for EBPR. It was found that a significant fraction of COD in the ozonolysate (36% of the COD) was biodegradable and the P-removal efficiency was about half that of acetate while N-removal efficiency was comparable to acetate. Kim et al. (2009) reported the solubilization of secondary sludge using H_2O_2 treatment. The solubility (sCOD/TCOD)

increased with increasing peroxide dosage. At a dosage of 1.6M H₂O₂, total solid reduction of 35% and solubility of approximately 50% was achieved.

While the above-mentioned processes are efficient for carbon recovery from undigested sludge, their capacity to recover carbon from digested sludge is limited. Thermal hydrolysis treatment was found to be an effective method for solubilization of both digested and undigested sludge readily biodegradable carbon (Haraguchi et al., 2006; Shanableh, 2000). However, this process is typically operated at high temperature and pressure which result in higher operational cost and capital investment. Recently, a relatively low temperature thermal-alkaline hydrolysis (Temperature:70-75°C, pH: 9.5-10, pressure: 1 atm) process (Lystek®) has been reported for solubilization of readily biodegradable carbon of raw as well as digested sludge (Singh et al., 2016). The Lystek product was reported to have 40%-50% of COD as soluble COD (sCOD) and an order of magnitude higher VFAs (10-15 g/L) compared to traditional biosolid treatment processes. Singh et al. (2016) reported initial SDNR rate for Lystek on methanol and glycerol acclimated biomass of about 0.03-0.06 g NO_x-N/gVSS.d and the denitrification rate with Lystek were mostly higher than primary effluent, methanol, and glycerol.

The aim of this study was to evaluate the potential of municipal biosolids treated by Lystek® process as a source of carbon for enhanced biological phosphorus removal from municipal wastewater. A control reactor with synthetic supplemental carbon source was also operated and its performance was compared with the biosolids fed reactor. In line soluble phosphorus concentrations were measured in order to evaluate the kinetics of phosphorus transformation.

2.Materials and methods

2.1 Sludge and wastewater

Thermal-alkaline hydrolyzed biosolids were obtained from Lystek International, Cambridge, ON, Canada. The biosolids were composed of primary sludge (PS) and waste activated sludge (WAS). The hydrolytic treatment was conducted at pH 9.5-10, temperature 70-75°C and atmospheric pressure at Lystek International Inc. Singh et al. (2016) reported the detailed characteristics of Lystek biosolids. The Lystek product contains 40%-50% of total chemical oxygen demands as soluble COD and an order of

magnitude higher VFA concentration (10 to 15 g/L) compared to the regular fermented biosolids (1 to 3 g/L) (Ji and Chen, 2010; Singh et al., 2016; Tong and Chen, 2007). The COD/N and COD/P ratios in Lystek are 16 and 25, respectively which makes Lystek an attractive carbon source for nutrient removal. In addition, calcium, potassium, sulfur, and magnesium concentrations in Lystek are 5, 6, 2, and 1 g/L, respectively (Singh et al., 2016). These can be supplementary to the inorganic nutrient requirement for microorganisms. In this study, the diluted filtrate of Lystek biosolids was used as a source of carbon in order to facilitate the feeding of Lystek into the reactor using lab-scale peristaltic pumps. In full-scale operation, dilution can be avoided through selection of proper slurry pumps. Lystek filtrate was obtained by diluting the Lystek biosolids by 10 times followed by centrifugation at 10,000 r/min (Beckman Coulter J2-HS) and filtration through 1.2 μ m filter paper (VWR glass fiber filter grade 696). Primary effluent (PE) was used as influent to the reactors and obtained from the Adelaide wastewater treatment plant, London, Canada and Calumet Water Reclamation Plant (WRP), Illinois, USA. In both wastewater treatment plants, primary clarification is typically enhanced by addition of chemicals, such as ferric chloride, alum, etc., where 30%-40% of the influent phosphorus .

2.2 Analytical methods

All chemicals used were analytical grades and obtained from Sigma-Aldrich. Both Lystek biosolids and influent wastewater were characterized with standard methods prior to testing. The wastewater and biosolids were stored at 4°C before prior to use. TSS, VSS, and alkalinity were measured using methods APHA 2540D, 2540E, and 2320B, respectively. Water quality parameters were measured using the following HACH methods: COD (HACH 8000), total nitrogen (HACH10072), ammonia (HACH10031), nitrate (HACH 10020), nitrite (HACH 10019), reactive phosphorus (HACH 8114), and total phosphorus (HACH 10127). VFA fractionation of Lystek biosolids was conducted on the soluble fraction. Lystek was diluted 10 times and filtered through 1.2 and 0.45- μ m filter paper for VFA analysis. The concentrations of different volatile fatty acids (VFAs) were analyzed using a gas chromatograph (Varian 8500, Varian Inc., Toronto, Canada) with a flame ionization detector equipped with a

fused silica column (30 m × 0.32 mm). Helium was used as the carrier gas at a flow rate of 2 mL/min. The injector temperature was set at 200°C with a split ratio of 5:1. The oven temperature was programmed at 80°C for 1 min, then a 20°C/min rate until 130°C, holding for 2 min, and then a 20°C/min rate until 165°C holding for 2 min. The detector temperature was set at 280°C. Phosphoric acid was used to adjust the pH of filtered samples to less than 2 prior to VFA analysis. Routine VFA analysis of diluted Lystek filtrate were performed using HACH method (TNT872) which measures the acetate and propionate fractions only.

2.3 Sequencing Batch Reactors operation

The experimental study was conducted using two sequencing batch reactors (SBRs) with a working volume of 2 L, fitted with diffused aeration system. The filling ratio was maintained between 50% to 60% with 3 to 4 cycles per day. The airflow rate was maintained between 0.2-0.4 L/min in order to maintain aerobic dissolved oxygen concentration in the range of 3-4 mg/L. The solid retention time (SRT) was maintained to 10 days via wasting at the end of aerobic stage. The pH of the system was recorded to be between 7.5-7.8 without active control. In order to facilitate the dosage of Lystek to the reactor, the biosolids were diluted 10 times, and filtered through 1.2-micron filter paper. The filtrate was added to the reactor as an alternative carbon source. Both SBRs were inoculated with recycled activated sludge from Adelaide Wastewater Treatment Plant. For each SBR, influent wastewater and supplemental carbon were fed separately using 2 different pumps to prevent the potential biodegradation of the synthetic carbon (reactor 1) and Lystek (reactor 2) in the wastewater tank.

The SBRs were operated with the following operational sequence: filling, anaerobic, aerobic, settling, and decanting. They were operated for 333 days with 4 distinct phases. In phase 1, the reactors were operated with VFA enriched synthetic wastewater to facilitate the initial growth of phosphorus accumulating organisms (PAOs). The chemical composition of the wastewater was as follows: COD (50:50 glucose:acetate) 300 mg/L, PO₄-P 5 mg/L, NH₄-N 24 mg/L, alkalinity (as CaCO₃) 340 mg/L, and trace metals (mg/L): MgSO₄ (69.6), CuSO₄·5H₂O (0.06), MnCl₂·4H₂O (0.24), CoCl₂·6H₂O (0.24), and ZnCl₂ (0.3). The remaining phases were operated with municipal wastewater without

(phase 2) or with supplemental carbon sources (phases 3 and 4). Table 3.1 summarizes the operating conditions for the SBRs.

Table 3.1 Reactors' operating conditions

	Phase 1	Phase 2	Phase 3	Phase 4
Working volume (L)	2	2	2	1.6
Filling ratio	50%	60%	60%	50%
Number of cycles/day	4	4	4	3
Aeration rate (L/min)	0.4	0.4	0.4	0.2
SRT (day)	10	10	10	10
Cycle schedule, min (filling, anaerobic, aerobic, settling, decanting)	30, 90, 120, 90, 30	30, 90, 165, 45, 30	30, 90, 165, 45, 30	30, 120, 240, 60, 30

3 Results and discussion

3.1 Wastewater and Lystek biosolids characteristics

Appendix A Tables S1a and S1b show the wastewater characteristics for the different phases of operation. The wastewater characteristics were comparable for the different phases of operation except for phase 4 where the wastewater strength was relatively lower compared to the other phases. The COD:P ratio ranged from 40 to 50, and 35 to 55 for Adelaide and Stickney wastewater, respectively. Similarly, COD:N ratio ranged from 5 to 6, and 5 to 9 for Adelaide and Stickney wastewater, respectively. It should be noted that both municipal wastewaters were lacking VFA and would require supplemental carbon to facilitate the PAO activity. Appendix A Tables S2a and S2b present the characteristics of Lystek biosolids. It can be observed that a significant fraction of soluble COD is short chain VFAs and nearly 30% of the VFAs are readily available (acetate/propionate) for phosphorus accumulating organisms (PAOs). As seen in Appendix A Tables S2a, the COD: P ratio (98) and COD: N ratio (12) for Lystek filtrate were found to be significantly higher than the primary effluent from both wastewater treatment plants, which makes Lystek an attractive carbon source in BNR.

3.2 Effluent quality and reactors' operational performance

During Phase 1 of operation, the reactors were fed with VFA enriched synthetic wastewater. Effluent phosphorus concentration was maintained as low as 0.1 mg/L (Figs. 3.1 and 3.2).

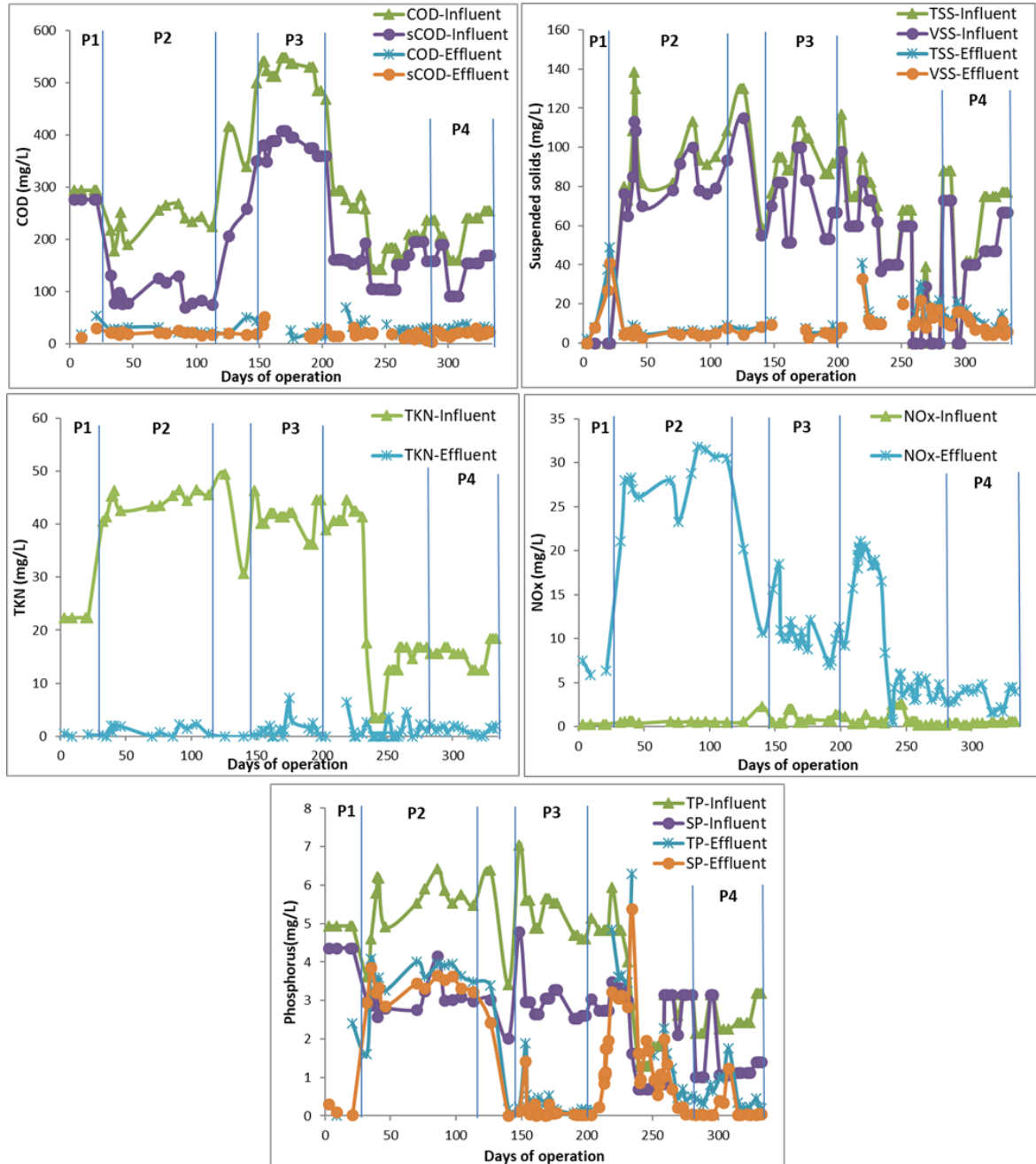


Figure 3.1 Influent and effluent characteristics-reactor 1

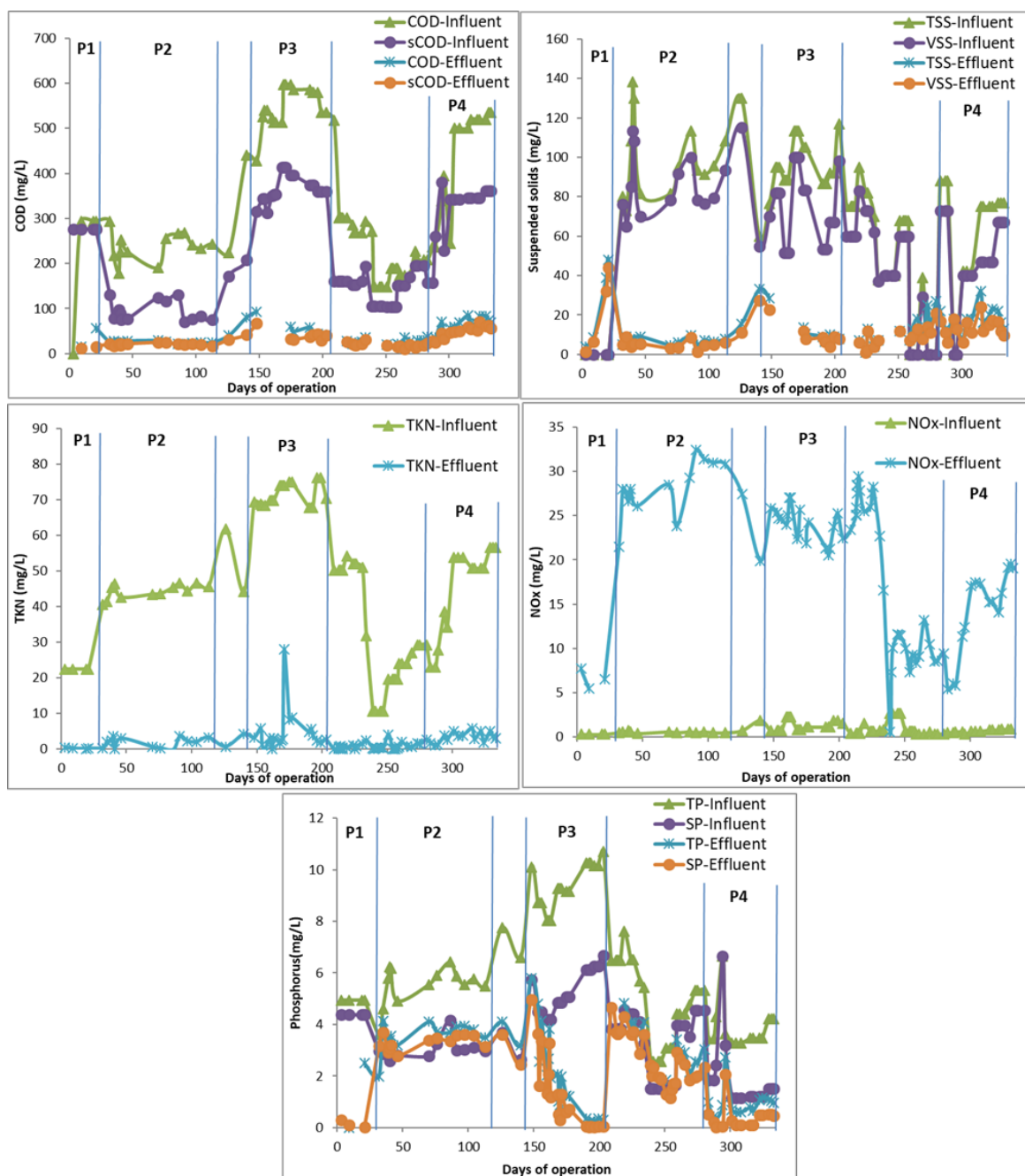


Figure 3.2 Influent and effluent characteristics-reactor 2

The P-removal efficiency of both reactors was found to be about 97% (Fig. 3.3). Taking into account the biomass yield of 0.28 g VSS/g COD and P-content of ordinary heterotrophs (1.5% - 2%), 6 out of 19 mg $\text{PO}_4\text{-P/day}$ was removed by biomass synthesis, clearly signifying EBPR in the system. The high P-removal performance is attributed to the abundant readily biodegradable carbon source as reflected by PAO accounting for about 68% of the P-removal.

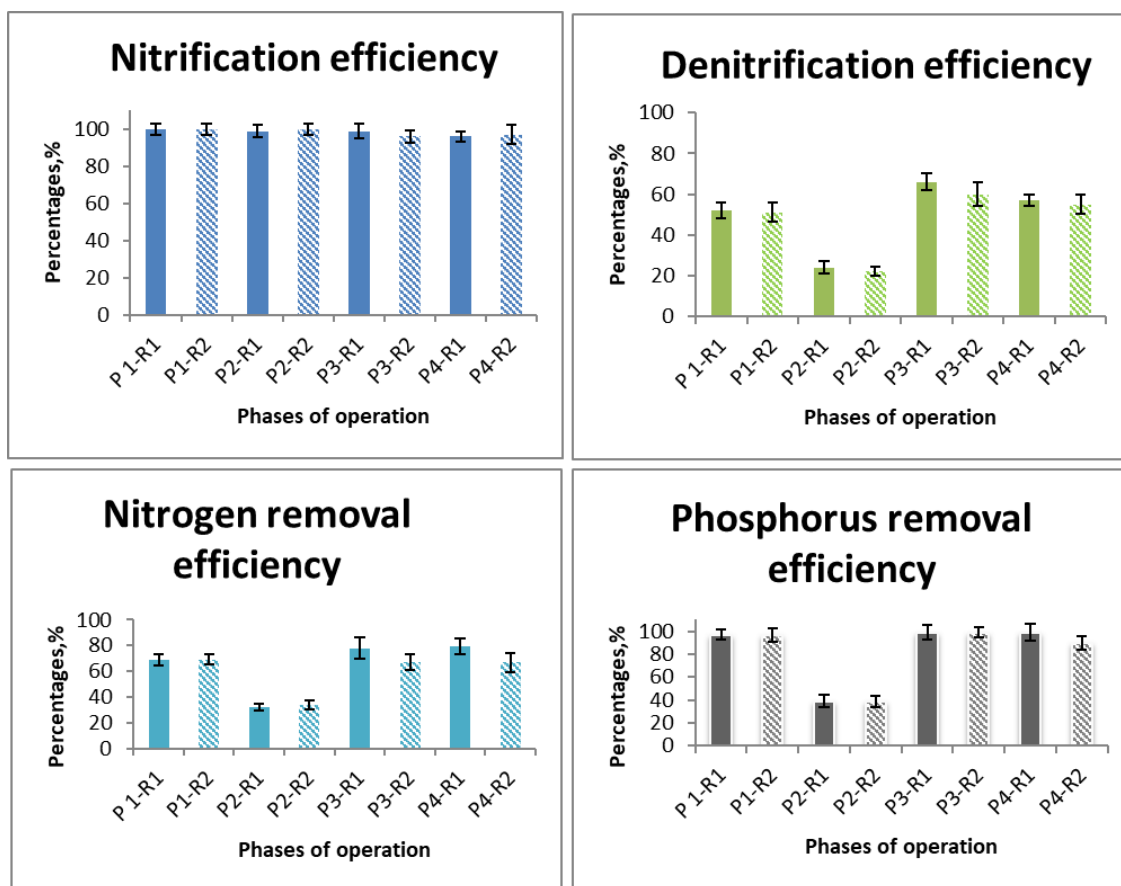


Figure 3.3 Nitrogen and phosphorus removal performances

After accounting for the nitrogen in biomass synthesis, it was found that almost complete nitrification was achieved with an effluent TKN concentration of 0.2-0.3 mg/L (Figs. 3.1 and 3.2). The TN removal efficiency was found to be approximately 70% in both reactors. The denitrification efficiency was about 50% with a COD/NO₃-N ratio of 4.7. Although sufficient COD was provided into the SBR influent, the low denitrification efficiency is due to the operational sequence of the SBR which denitrified following the decant phase during the anoxic fill with a filling ratio of 50%. Effluent sCOD and suspended solids were maintained at 15-20 mg/L (Figs. 3.1 and 3.2).

In phase 2, the synthetic wastewater was switched to municipal wastewater from Adelaide WWTP. The EBPR performance rapidly dropped with an effluent SP concentration of 3.5- 4 mg/L (Figs. 3.1 and 3.2) and P-removal efficiency of 39% (Fig. 3.3). The TN removal and denitrification efficiencies also decreased to about 33% and 23%, respectively, although the nitrification efficiency was as high as 100%. Incomplete denitrification also led to NO_x-N accumulation in the reactors which increased the

effluent $\text{NO}_x\text{-N}$ concentration to 25-30 mg/L (Figs. 3.1 and 3.2). These results clearly signify the lack of readily biodegradable carbon for PAOs as well as denitrifying bacteria during phase 2 of operation.

In order to supplement the lack of readily biodegradable carbon, an external carbon source (R1: synthetic VFA, R2: Lystek filtrate) was added to the primary effluent in phase 3. Prior to phase 3, a carbon dosage optimization was attempted. Additional carbon dosages of 220 and 120 mg sCOD/L were found to be insufficient to maintain a consistent effluent $\text{TP} < 1$ mg/L and $\text{SP} < 0.5$ mg/L in reactor 2 (Fig. 3.2). However, in reactor 1, a dosage of 220 mg sCOD/L was able to maintain a $\text{TP} < 1$ mg/L and $\text{SP} < 0.5$ mg/L (Fig. 3.1). In phase 3 of the reactor operation, with an additional carbon dosage of 310 mg sCOD/L, the effluent phosphorus concentration was maintained at $\text{TP} < 1$ mg/L and $\text{SP} < 0.5$ mg/L for more than 3 SRT turnover (Figs. 3.1 and 3.2) for both reactors. Nearly 100% and 90% of the steady-state data satisfied such effluent characteristics for reactors 1 and 2, respectively. The P-removal efficiency also increased to 99% for both reactors. After accounting for P-removal via biomass synthesis for biomass yield of 0.22 and 0.27 gVSS/gCOD, about 59% and 78% of the P-removal was performed by PAOs for reactors 1 and 2, respectively. The PAO activity in phase 3 was comparable to phase 1. TN removal efficiencies were 78% and 67% for reactors 1 and 2, respectively. The nitrification efficiencies for reactors 1 and 2 were 99% and 96%, respectively; while the denitrification efficiencies were about 66% and 60%, respectively. Effluent sCOD and suspended solids were maintained at less than 20 mg/L for reactor 1. However, effluent sCOD (40-60 mg/L) were slightly higher for reactor 2. The effluent characteristics and nutrient removal performance in phase 3 confirm the availability of readily biodegradable carbon in Lystek for N and P removal from municipal wastewater.

In phase 4, Lystek was supplemented to a low strength municipal wastewater from the Calumet WRP, Illinois, USA. Prior to phase 4, a carbon dosage optimization was also attempted. Additional carbon dosages of 60 and 90 mg sCOD/L were found to be insufficient to maintain a consistent effluent $\text{TP} < 1$ mg/L and $\text{SP} < 0.5$ mg/L in reactor 2 (Fig. 3.2). However, in reactor 1, a dosage of 90 mg sCOD/L was able to maintain a $\text{TP} < 1$ mg/L and $\text{SP} < 0.5$ mg/L (Fig. 3.1). In Phase 4, with an additional carbon dosage (R1: 90 mg sCOD/L, R2: 310 mg sCOD/L) the reactors' effluents were maintained at $\text{TP} < 1$ mg/L

and $SP < 0.5$ mg/L (Figs. 3.1 and 3.2). Nearly 100% and 80% of the steady-state data satisfied such effluent characteristics for reactors 1 and 2, respectively. As shown in Fig. 3.3, the P-removal efficiencies were 99% and 90% for reactors 1 and 2, respectively. Considering, biomass yield of 0.35 and 0.32 gVSS/gCOD, about 57% and 35% of the P was removed by PAOs in reactor 1 and 2, respectively. A lower percentage of P-removal via PAOs in reactor 2 is a consequence of the higher organic loading rate (330 and 700 mgCOD/L.d in reactor 1, and 2) contributing to $P_{\text{synthesis}}$ of 2.8 and 5.3 mg $PO_4\text{-P}$ /day for reactor 1 and 2, respectively. The nitrification efficiency was 96% and 97% in reactors 1 and 2, respectively. The denitrification efficiency was 57% and 56% for reactors 1 and 2, respectively. TN removal efficiencies were 79% and 67% for reactors 1 and 2, respectively.

In phases 3 & 4, due to additional nitrogen contribution from Lystek, effluent $NO_x\text{-N}$ concentration in reactor 2 (Lystek) was as high as 22 and 17 mg/L in phases 3 and 4, respectively. This placed denitrifiers in advantageous position for carbon consumption compared to PAOs, as denitrification occurred prior to VFA uptake by PAOs. In order to overcome this, a high sCOD supplementation (+310 mg sCOD/L) was required to facilitate PAO activity in the reactors. While such a high nitrate concentration can be problematic for SBR operation, this can be overcome in continuous-flow systems, such as A_2O , MUCT, etc. by maintaining a low nitrate concentration prior to the anaerobic stage, thereby facilitating the PAO accumulation in the biomass without excessive carbon supplementation.

3.3 Nitrogen and phosphorus mass balance

Nitrogen mass balance in the SBR were performed using Eqs. (3.1)-(3.5). Eq. (3.1) was used to determine the input-N (Influent-N, mg/day) to the SBR.

$$\text{Influent-N} = Q \times (C_{\text{Inf-TKN}} + C_{\text{Inf-NO}_x}) \quad (3.1)$$

Where, Q (L/day) and C (mg/L) represents the flow and concentration, respectively.

The influent nitrogen to the reactor is primarily transformed via two pathways: (1) nitrification/denitrification, and (2) cell synthesis. The output-N (Effluent-N, mg/day) from the reactor is calculated from following equations:

$$\text{Effluent-N} = N_{\text{CE}} + N_{\text{DN}} + N_{\text{WAS}} \quad (3.2)$$

$$N_{\text{CE}} = Q \times (C_{\text{Eff.-sTKN}} + C_{\text{Eff.-NO}_x} + f_N \times C_{\text{Eff.-VSS}}) \quad (3.3)$$

$$N_{\text{DN}} = Q \times (C_{\text{Inf-TKN}} - C_{\text{Eff.-sTKN}} - C_{\text{N-cell synthesis}} - C_{\text{Eff.-NO}_x}) \quad (3.4)$$

$$N_{\text{WAS}} = (C_{\text{MLVSS}} \times V_R / \theta_C - Q \times C_{\text{Eff.-VSS}}) \times f_N \quad (3.5)$$

Where, N_{CE} (mg/day), N_{DN} (mg/day), N_{WAS} (mg/day), f_N , V_R (L), θ_C (day) represents the nitrogen in the clarified effluent, denitrification, waste activated sludge, N-content of the biomass, reactor volume, solid retention time, respectively. The value of θ_C was maintained at 10 days throughout the study. The value of f_N was measured experimentally and found to be between 10% to 12%.

Eqs. (3.6)-(3.9) were used for performing the phosphorus mass balance. The influent phosphorus (Influent-P, mg/day) is the sum of soluble ortho-phosphorus and particulate phosphorus.

$$\text{Influent-P} = Q \times C_{\text{Inf-TP}} \quad (3.6)$$

$$\text{Enfluent-P} = P_{\text{CE}} + P_{\text{WAS}} \quad (3.7)$$

$$P_{\text{CE}} = Q \times (C_{\text{Eff.-SP}} + f_P \times C_{\text{Eff.-VSS}}) \quad (3.8)$$

$$P_{\text{WAS}} = (C_{\text{MLVSS}} \times V_R / \theta_C - Q \times C_{\text{Eff.-VSS}}) \times f_P \quad (3.9)$$

Where, P_{CE} (mg/day), P_{WAS} (mg/day), f_P represents phosphorus in the clarified effluent, waste activated sludge, and the P-content of biomass, respectively. The experimental value for f_P were found to be between 2.8%-3.8% and 3.2%-5.4% for reactors 1 and 2, respectively.

Table 3.2 shows the distribution of influent nitrogen across various process streams. In phase 2, the majority (66%-68%) of the influent nitrogen ended up in the clarified effluent as $\text{NO}_x\text{-N}$. Approximately 20% of the influent nitrogen was denitrified and the remaining nitrogen was either nitrified or consumed in the cell synthesis. Overall nitrogen removal was found to be 33% and 23% for reactors 1 and 2, respectively. Only about 20% of the total available COD was used for denitrification. This clearly shows the lack of biodegradable carbon in the influent wastewater for denitrification. In phase 3, reactors 1 and 2 were supplemented with synthetic carbon and Lystek, respectively.

Table 3.2 Distribution of influent-N across various effluent streams (percentages of total influent-N)

Run	Phase 2		Phase 3		Phase 4	
	Reactor 1	Reactor 2	Reactor 1	Reactor 2	Reactor 1	Reactor 2
N-WAS	12%	10%	34%	22%	44%	15%
N-CE	66%	68%	22%	33%	25%	36%
N-denitrified	20%	19%	40%	43%	25%	38%
N-balance	-2%	+3%	-4%	-2%	6%	11%

WAS: waste activated sludge; CE: clarified effluent

As shown in Table 3.2, the percentage of denitrified-nitrogen nearly doubled signifying excellent activity of the denitrifying bacteria. A significant fraction of the influent-N was also partitioned into the biomass and left the system with the waste activated sludge. The overall nitrogen removal was found to be 78% and 67% for reactors 1 and 2, respectively. Approximately 17% and 31% of the total available COD was utilized for denitrification for reactors 1 and 2, respectively. Similar results were obtained in phase 4 when the reactors were operated with low strength primary effluent from Calumet wastewater treatment plant. This indicates that the denitrification potential of Lystek was comparable to synthetic VFAs.

Table 3.3 shows the partitioning of the total influent phosphorus into clarified effluent and biomass. In phase 2, due to lack of VFAs in the influent wastewater limited biological phosphorus removal was observed.

Table 3.3 Distribution of influent-P across various effluent streams (percentages of total influent-P)

Run	Phase 2		Phase 3		Phase 4	
	Reactor 1	Reactor 2	Reactor 1	Reactor 2	Reactor 1	Reactor 2
P-WAS	33%	27%	88%	80%	89%	65%
P-CE	64%	64%	4%	5%	9%	23%
P-balance	3%	9%	8%	15%	2%	12%

WAS: waste activated sludge; CE: clarified effluent

More than 60% of the influent phosphorus ended up in the clarified effluent for both reactors resulting in a net phosphorus removal efficiency of only about 40%. Approximately, 10% of the total available COD was used by PAOs for phosphorus removal. In phase 3, when supplemented with external carbon, both the reactors showed a net phosphorus removal efficiency of 99% and more than 80% of the influent phosphorus was accumulated in the biomass for both reactors. Approximately 10% and 20% of the total available COD was used by the PAOs in reactors 1 and 2, respectively. This clearly implies enhanced biological phosphorus removal in both reactors. Similar EBPR performance was also observed in phase 4. In all phases of operation, Lystek was found to have comparable EBPR performance with synthetic VFAs.

The nitrogen and phosphorus mass balances for reactors 1 and 2 are shown in Tables 3.4 and 3.5, respectively. The mass balance was well accounted for within a 5%-15% margin of error.

Table 3.4 Nitrogen mass balance for reactor 1 and reactor 2 (units in mg/day)

	Input-N						Output-N					
Run	Phase 2		Phase 3		Phase 4		Phase 2		Phase 3		Phase 4	
	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2
TKN	220	220	192	344	36	128						
sTKN							1.2	0	0.7	10	0.7	2.4
NO _x -N	2.4	2.4	4	6	1	2	149	149	43	106	7.2	41
WAS-N							26	22	73	80	16.4	20
Effluent VSS-N							2.2	2.4	2.4	4	1.5	4
N-denitrified							47	43	84	158	9.5	49
Closure= (Input- N/Output- N)×100%	99%	102%	97%	98%	105%	111%						

Table 3.5 Phosphorus mass balance for reactor 1 and reactor 2 (units in mg/day)

	Input-P						Output-P					
Run	Phase 2		Phase 3		Phase 4		Phase 2		Phase 3		Phase 4	
	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2
Total-P	27.2	27.2	23.3	48.6	6.6	9.1						
Soluble-P							16.7	16.5	0.2	0.7	0.1	0.9
WAS-P							8.9	7.4	20.6	39.1	5.9	5.9
Effluent VSS-P							0.78	0.82	0.7	1.8	0.5	1.2
Closure (Input- P/Output- P)×100%	103%	110%	108%	116%	102%	113%						

Table 3.4 shows the amount of different types of nitrogen in the influent and effluent streams. In phases 3 and 4, the influent nitrogen loading into reactor 2 (Lystek reactor) was significantly increased. As Lystek contains about 4 g/L of SN, this was primarily due to the excessive nitrogen contribution (approximately 40% of the influent nitrogen) from the Lystek filtrate. The increased NO_x-N concentration in the effluent as

well as increased denitrification confirmations that nitrification was not affected and was nearly complete.

Table 3.5 shows the amount of phosphorus in various process streams in the SBRs. The phosphorus mass balance was well accounted for within less than 15% margin of error. The particulate phosphorus content of Lystek biosolids was 4.6% and as the Lystek filtrate was obtained through 1.2- μm filter paper, some of the particulate phosphorus from Lystek contributed to the influent total phosphorus. The phosphorus contribution from Lystek filtrate was more than 50% and 25% of influent TP for phases 3 and 4, respectively. The P-content of the biomass in the Lystek reactor (5.4%) was also found to be higher than that of synthetic VFA reactor (2.9%). The higher margin of error and high P-content of the biomass for Lystek reactor could possibly due to the non-biodegradable particulate phosphorus contribution from Lystek.

3.4 Kinetics of phosphorus release and uptake in the SBRs

During phase 4, inline cyclic tests for both reactors were conducted in order to compare the specific P-release/uptake rates of the biomass for reactors 1 and 2. Fig. 3.4 shows the P-release and uptake profiles in the reactors.

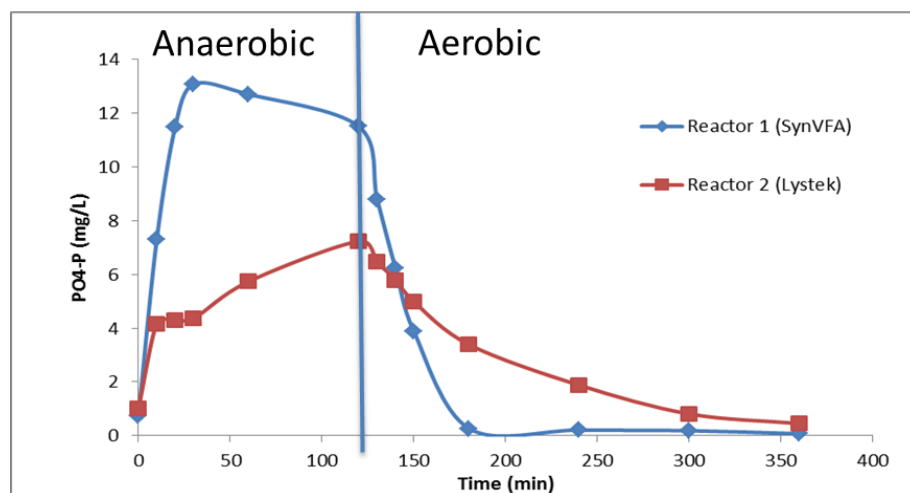


Figure 3.4 Phosphorus release and uptake profile in the reactors

As can be seen from Fig. 3.4, the kinetics of phosphorus release and uptake in reactor 2 (LysVFA) are significantly slower compared to reactor 1 (SynVFA). This can be primarily for 2 reasons: (1) competition from denitrifiers, (2) complexity of the carbon source. As previously mentioned, there was an excess nitrogen contribution from Lystek

which increased the competition for carbon between PAOs and denitrifiers. The initial NO_x-N concentration in reactor 2 was as high as 11 and 8.5 mg/L in phases 3 and 4, respectively; which is significantly higher than reactor 1 (4.5 and 1.5 mg/L in phases 3, and 4, respectively). As seen from Appendix A Table S1a, after the initial degradation of acetate and propionate by the denitrifiers, enough acetate and propionate (280 and 80 mg/L in phases 3 and 4, respectively) are available for PAOs. However, as seen in Appendix A Table S1b, after accounting for acetate and propionate for denitrification, little to no acetate and propionate are available for PAOs. Therefore, the anaerobic P-release in reactor 2 was primarily driven by higher order VFAs (butyric and valeric acid). It has been also reported in the literature that butyrate uptake rate of PAOs is 70% slower to that of acetate and propionate (Pijuan et al., 2009). Carbon recovery ratio (PHA produced/substrate uptake) with butyrate was also found to be 40% to 50% lower than acetate and propionate. Since 71% of LysVFA is composed of butyric and valeric acid, the slower kinetics in reactor 2 are expected. As seen in Fig. 3.4, the P-release did not reach a plateau within the 2 hour anaerobic contact time, thus indicating the incomplete utilization of carbon in the anaerobic stage. Therefore, the slower kinetics and incomplete utilization of carbon in the anaerobic stage resulting in higher concentration of externally available carbon which must be consumed before PAOs can consume polyhydroxyalkonates (PHA) as a source of carbon. This led to slower P-uptake in the aerobic stage in reactor 2.

Table 3.6 shows the P-release and uptake rates of the biomass in the reactors. As evident in Table 3.6 that specific P-release/uptake rate for reactor 2 (Lystek) is approximately one-third of the reactor 1 (SynVFA) which signifies a large difference in PAO activity between the reactors.

Table 3.6 Specific phosphorus release and uptake rates

	Reactor 1 (Synthetic VFA)	Reactor 2 (Lystek)	Reactor 2 (Lystek, normalized to acetate-propionate only)
SP. P-release rate (mg PO ₄ -P/g VSS·h)	19 ± 6.2	6 ± 3.7	25 ± 15.7
SP. P-uptake rate (mg PO ₄ -P/g VSS·h)	8 ± 1.7	2.5 ± 0.83	10 ± 3.5

As previously mentioned, this is understandable as the synthetic carbon sources, such as acetate and propionate are the known pure and easily biodegradable carbon sources compared to the natural sources of carbon or complex VFAs, such as Lystek. The specific phosphorus release (SPRR) and uptake (SPUR) rates with SynVFA were 19 and 8 mg P/g VSS.hr, respectively and with LysVFA were 6 and 2.5 mg P/g VSS.hr, respectively (Table 3.6). For the Lystek reactor, both SPRR and SPUR were relatively lower compared to the typical values reported in the literature (SPRR: 5-32.5 mg PO₄-P/g VSS.hr; SPUR 5.7-20.8 mg PO₄-P/g VSS.h) (Mamais and Jenkins, 1992; Kuba et al., 1997; Brdjanovic et al., 1998; Monti et al., 2007). However, it should also be noted that majority of the literature reported SPRR and SPUR using synthetic carbon sources (primarily acetate and propionate) in contrast to the complex carbon source such as in a product derived from a WWTP biosolid, e.g. Lystek process where butyric acid and valeric acid are the dominant VFAs. Table 3.6 also shows the P-release/uptake rates for reactor 2 when normalized with respect acetate-propionate concentration (by dividing the overall rate with acetic acid plus propionic acid content of LysVFA) in Lystek. It can be clearly seen the normalized rates are very similar to ones obtained with synthetic carbon supplementation in reactor 1. This further indicates that higher order VFAs (C4 and higher) in Lystek were primarily responsible for slower kinetics in reactor 2.

3.5 Implication of Lystek process in full-scale EBPR plants

This study showed that by dosing Lystek filtrate into primary effluent, effluent phosphorus limits can be met without supplemental synthetic carbon sources. It is particularly important to know whether recirculation of internal carbon via the Lystek process can mitigate carbon requirement for full scale enhanced biological phosphorus removal process. Fig. 3.5 shows a simplified block diagram for an EBPR plant (with conventional rather than chemically enhanced primary treatment) with integrated Lystek process (calculation shown in Appendix A).

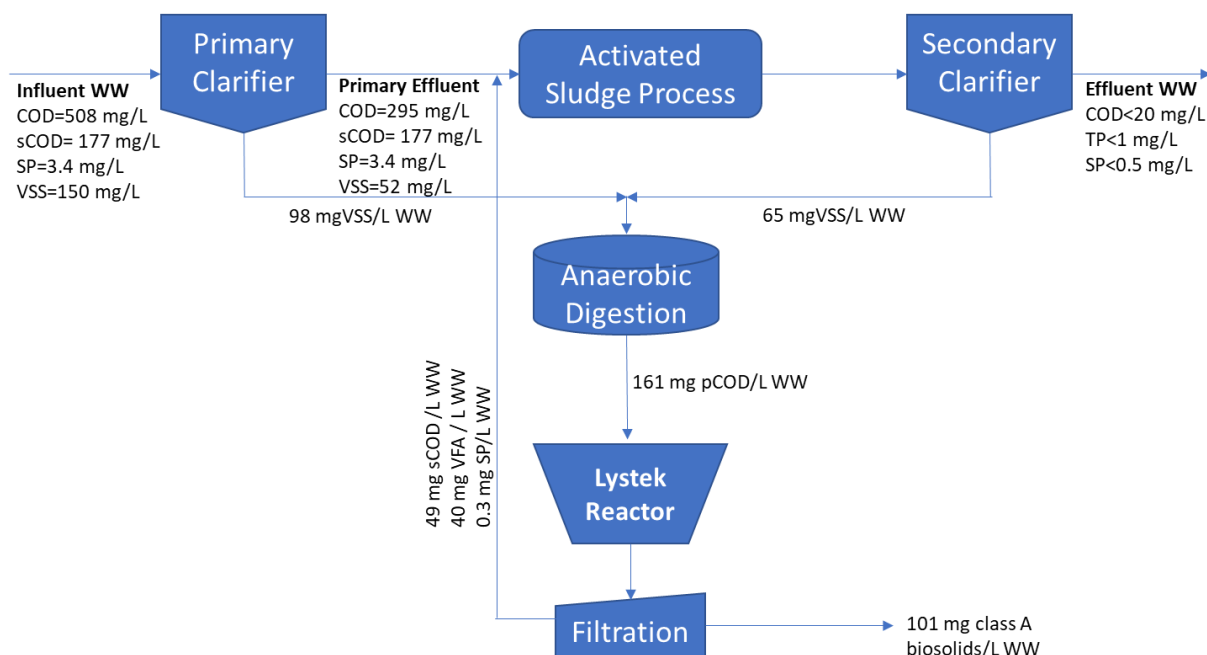


Figure 3.5 Enhanced biological phosphorus removal with integrated Lystek process for a typical medium strength wastewater(Metcalf and Eddy, 2014)

Using wastewater characteristics presented in Appendix A Table S3a, a sample calculation has been conducted to show the feasibility of Lystek biosolid filtrate as a carbon source in full-scale wastewater treatment plants. As shown in Fig. 3.5, for a typical medium strength municipal wastewater with COD 508 and VSS 150 mg/L, by maintaining 65% VSS removal in primary clarification and 10 day SRT in the biological system, 98 mg VSS as primary sludge and 65 mg VSS as waste activated sludge would be produced per litre of wastewater treated. Considering 50% and 40% VSS destruction for PS and WAS during anaerobic digestion and pCOD/VSS ratio of 2 and 1.6 for anaerobically digested primary and secondary sludge(WEF, 2010), 161 mg pCOD would be fed to the Lystek reactor per litre of wastewater treated. Combined high shear mixing, temperature and alkaline condition can contribute to a 25%-30% pCOD solubilization in the Lystek reactor, as evident from the Lystek characteristics in Appendix A Table S2a. Assuming, 30% pCOD solubilization in Lystek reactor, 40 mg VFA can be produced per litre of wastewater treated. With complete internal recirculation of Lystek filtrate, an influent VFA to soluble phosphorus ratio of 11 can be maintained. In EBPR practice, the typical values for mg VFA required/mg P are between 10 to15. Therefore, Lystek

biosolids filtrate should mitigate the carbon requirement for enhanced biological phosphorus removal process in typical municipal wastewater treatment plant.

3 Conclusions

In general, Lystek biosolids filtrate were found to be an alternate carbon source for phosphorus and nitrogen removal from municipal wastewater due to the presence of high amount of soluble COD and VFAs. The extent of PAO activity largely relied on the readily biodegradable fraction of the Lystek biosolids. Higher order VFAs (C4 or higher) were found to be contributing to the EBPR; however, with a slower kinetics than that of acetate and propionate. As a result, a higher dosage may be necessary to improve the COD:N:P ratio and higher initial acetate/propionate concentration in order to achieve low effluent P level. It was found that NO_x-N concentration in the anaerobic stage dictates the EBPR kinetics in mixed VFA system when acetate and propionate availability is limited. In spite of the nitrogen and phosphorus contribution from Lystek product, the effluent phosphorus concentrations were maintained at TP<1 mg/L and SP<0.5 mg/L. This study confirms the effectiveness of using Lystek biosolids filtrate, a naturally derived and sustainable carbon source from a municipal WWTP for enhanced biological phosphorus removal.

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Chapter 4

**Impact of Dissolved Oxygen
Concentration and DPAOs: Nitrifiers
Population Ratio on Nutrient Removal in
the EBPR Process**

1.Introduction

Enhanced biological phosphorus removal (EBPR) is a sustainable and environmentally friendly engineered wastewater treatment process that is capable of maintaining a low effluent phosphorus concentration. The fundamental principle of EBPR is the alternating anaerobic-aerobic or anoxic condition that promotes the growth of PAOs, which can store VFAs under anaerobic conditions and utilize them under aerobic or anoxic conditions along with phosphorus uptake (Adrian Oehmen et al., 2007).

Aeration is the most energy intensive operation in wastewater treatment plant and accounts for 45%-75% of plant energy cost (Gu et al., 2017). In recent years, optimizing aeration requirement has become an important task for municipal wastewater treatment plants. Recent technological developments are also more focused on low dissolved oxygen processes (Chen et al., 2014; Jimenez et al., 2014). In the conventional A2O process, high DO concentration in the aeration tank leads to greater oxygen contribution from nitrate recycle into the anoxic zone. This can favor the proliferation of denitrifying glycogen accumulating organisms (DGAOs) over denitrifying phosphorus accumulating organisms (DPAOs) and deteriorate biological phosphorus removal (Q. Yuan & Oleszkiewicz, 2011). While it is a common practice to maintain 2-3 mg/L of DO in the aeration tank for stable nitrogen and phosphorus removal, recent studies showed aerobic P-uptake is feasible at dissolved oxygen concentration below 1 mg/L (Jimenez et al., 2014).

This study investigates the impact of low dissolved oxygen concentration and DPAOs to nitrifiers population ratio on nutrient removal. The DPAOs enrichment process was carried out in a separate SBR, capable of utilizing both $\text{NO}_2\text{-N}$ and O_2 as an electron acceptor. NOB washout from the nitrifying sludge was obtained in a separate SBR operated under low DO condition.

2.Materials and Methods

2.1. Sludge and wastewater

Synthetic wastewater was used for DPAOs enrichment with the following characteristics; 160 mg/L COD (acetate), 8 mg/L $\text{NH}_4\text{-N}$, 6 mg/L $\text{PO}_4\text{-P}$, and trace metals

(70 mg/L MgSO_4 , 0.06 mg/L $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.24 mg/L $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 0.24 $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 0.3 mg/L ZnCl_2 . Partial nitrification SBR was operated with synthetic wastewater with following characteristics: 120 mg/L COD (acetate), 50 mg/L $\text{NH}_4\text{-N}$, 4 mg/L $\text{PO}_4\text{-P}$, 400 mg/L alkalinity (as CaCO_3), and trace metals at the same concentration as DPAOs SBR. Activated sludge inoculum was obtained from Greenway wastewater treatment plant, London, ON, Canada. The wastewater and inoculum were stored at 4°C prior to use.

2.2. Analytical methods

All chemicals used were analytical grades and obtained from Sigma-Aldrich. TSS, VSS, and alkalinity were measured using methods APHA 2540D, 2540E, and 2320B, respectively. Water quality parameters were measured using the following HACH methods: COD (HACH 8000), total nitrogen (HACH10072), ammonia (HACH10031), nitrate (HACH 10020), nitrite (HACH 10019), reactive phosphorus (HACH 8114), and total phosphorus (HACH 10127).

3. Results and discussions

3.1. DPAOs enrichment in mother SBR reactor

DPAOs enrichment in the mother SBR reactor was obtained according to the method outlined in the literature (Dai et al., 2017). A SBR with 2L effective working volume was operated with 50% filling ratio for 3 cycles per day. The operation sequence was as follows: 10 min fill, 90 min anaerobic, 180 min anoxic, 120 min aerobic, 70 min settling, and 10 min decant. The influent characteristics were as follows: 160 mg/L COD (acetate), 8 mg/L $\text{NH}_4\text{-N}$, 6 mg/L $\text{PO}_4\text{-P}$, and trace metals. In order to prevent nitrification during the aerobic phase 20 mg/L allylthiourea was also added in each phase. During the anoxic cycle, $\text{NO}_2\text{-N}$ was provided from a concentrated NaNO_2 solution using a chemical feed pump. In each cycle, 3 spikes of 5 mg/L $\text{NO}_2\text{-N}$ were added for the anoxic phosphorus uptake. Fig. 4.1 shows a typical cycle of operation in the mother SBR.

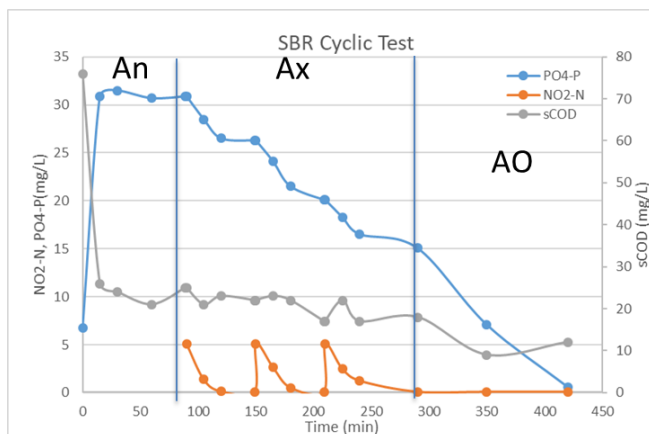


Figure 4.1 Variations of N, P, COD in a typical cycle in the mother SBR

As seen in Fig. 4.1, during the anaerobic phase most of the readily biodegradable carbon was utilized for synthesis of PHA. The sp. Phosphorus release rate was found to be 53 mgPO₄-P/g-VSS.hr (Table 4.1) which is higher than the typically reported values of 5-32 mgPO₄-P/g-VSS.hr in the literature (Brdjanovic et al., 1998; Kuba et al., 1997; Mamais & Jenkins, 1992; Monti et al., 2007).

Table 4.1 Kinetics of nutrient removal in mother DPAO- SBR

	An	Ax-1	Ax-2	Ax-3	AO
SPRR (mgPO ₄ -P/g-VSS.hr)	53				
SPUR (mgPO ₄ -P/g-VSS.hr)		4.9	5.3	4	
SDNR (mgNO ₂ -N /g-VSS.hr)		5.5	5.1	4.3	3.7
N-reduction rate/P-uptake rate		1.12	0.96	1.07	

In the anoxic phases, all the added NO₂-N was reduced along with phosphorus uptake. This confirms the enrichment of nitrite reductase enzyme in the DPAOs in the mother reactor. In both the anoxic and aerobic phases, specific P-uptake rate was found to be significantly lower compared to the reported values in literature. The typical values for aerobic P-uptake are reported to be between 5-20 mgPO₄-P/g-VSS.hr (Brdjanovic et al., 1998; Kuba et al., 1997; Mamais & Jenkins, 1992; Monti et al., 2007). This is primarily due to the lack of pH control during the anoxic phase. The increased pH 8.2 from anoxic cycle also significantly affected the aerobic P-uptake rate as seen in Table 4.1. The average N- reduced to P-uptake rate was found to be 1.05 which is lower than the typical reported values of 1.3-1.5 (Dai et al., 2017; Peng et al., 2011) which signifies slightly lower carbon utilization efficiency in the enriched DPAOs culture.

3.2. Partial nitrification at low DO

A 2L SBR was inoculated with returned activated sludge from Greenway WWTP and operated 3 cycles/day. Low DO (0.3-0.5 mg/L) coupled with short SRT (8 days aerobic SRT) facilitated the washout out of NOB and stable nitrite accumulation ratio, NAR (upto 85%). Fig.2 shows the operational performance of the partial nitrification reactor.

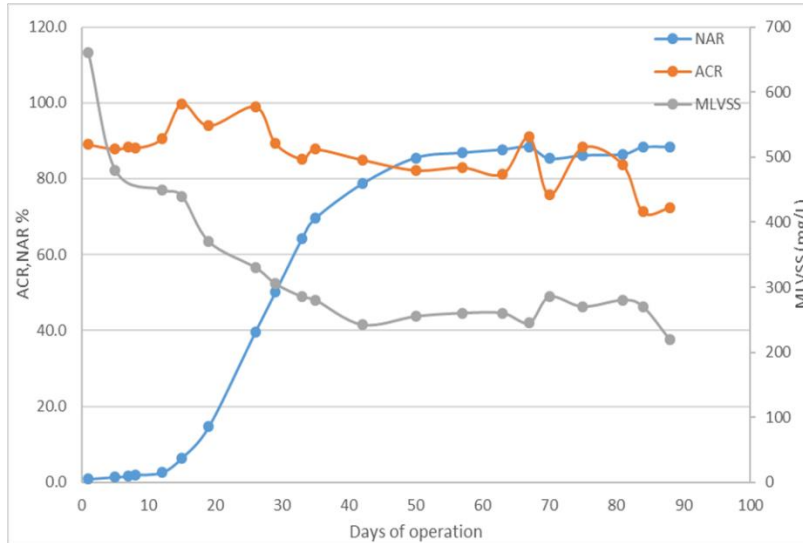


Figure 4.2 Operational performance of partial nitrification SBR operated at low DO (0.3-0.5 mg/L) and short SRT (8 days)

As shown in Fig. 4.2, no significant nitrite was accumulated in the effluent due to high NOB population in the first 2 weeks. At the start-up, the apparent sp. growth rates (25°C, DO 0.25 mg/L, and aerobic SRT 8 days) for AOB and NOB was calculated to be, 0.103 and -0.026d⁻¹, respectively. This is highly favorable for NOB washout and as seen in Fig. 4.2, after 2 weeks NOB washout started to take place and in about 2 months stable nitrite accumulation was achieved with NAR ranged from 80% to 85%. Ammonium conversion ratio (ACR) was found to be more than 80% throughout the period of study. The biomass concentration stabilized at 240 mg-MLVSS/L in about 40 days.

3.3. Batch study on nitrifiers and DPAOs mixed sludge at various nitrifying to DPAO sludge mass ratios

Batch studies were conducted with varying dissolved oxygen concentration and N-sludge (nitrifying sludge) to P-sludge (DPAO sludge) ratios. Both nitrifying and

DPAOs sludge was washed and centrifuged to make a concentrated stock. Concentrated N-sludge and P-sludge at specific ratios (1:1, 1:2, 1:4) was taken to a 250 mL conical flask and diluted to 250 mL with DI water. N, P, and COD was provided from concentrated stock solutions to provide an initial concentration of 25 mg NH₄-N/L, 3 mg PO₄-P, and 100 mg sCOD/L. Each batch study consists of 1 hour of anaerobic followed by 5 hour of aerobic contact time.

Scenario 1. Varying N-sludge to P-sludge ratio at 0.5 mg/L dissolved oxygen concentration

Three batch tests were conducted to study the impact of DPAOs on nitrification at varying nitrifiers to DPAOs population ratio. The N-sludge: P-sludge ratio tested with incremental DPAOs population was as follows: 1:1, 1:2, 1:4 on mass basis. Tables 4.2-4.4 show the initial, end of anaerobic phase and final effluent concentration in each of the batch tests.

Table 4.2 N, P, C transformation at N-sludge to p-sludge ratio 1:1

Concentration (mg/L)	Initial	Anaerobic Effluent	Final Effluent
NH ₄ -N	25	23.8	0.125
PO ₄ -P	3	11.1	3.54
sCOD	100	55	10
NO ₃ -N	0	0	6.6
NO ₂ -N	0	0	0.03

Table 4.3 N, P, C transformation at N-sludge to p-sludge ratio 1:2

Concentration (mg/L)	Initial	Anaerobic Effluent	Final Effluent
NH ₄ -N	25	24.1	5.25
PO ₄ -P	3	17	4.33
sCOD	100	40	14
NO ₃ -N	0	0	3
NO ₂ -N	0	0	0.15

Table 4.4 N, P, C transformation at N-sludge to p-sludge ratio 1:4

Concentration (mg/L)	Initial	Anaerobic Effluent	Final Effluent
NH ₄ -N	25	23.5	10.7
PO ₄ -P	3	22	7.24
sCOD	100	26	11
NO ₃ -N	0	0	1
NO ₂ -N	0	0	0.02

As seen in Table 4.2, DPAOs did not have any significant impact on nitrification at a N-Sludge: P-sludge ratio of 1:1. Most of the ammonium was oxidized and subsequently reduced by the ordinary denitrifiers as well as DPAOs. The ammonium conversion ratio (ACR) was about 99.5%. The residual NO₃-N concentration of 6.6 mg/L signifies DPAO's lack of capability to use nitrate as an electron acceptor. This is in agreement with the previous finding on DPAOs genome study which confirmed that DPAOs lacks the gene required for synthesis of nitrate reductase enzyme (Mchardy et al., 2006).

As the DPAOs population was increased in the sludge, ACR was reduced to 79% and 57% at a N-sludge :P-sludge ratio of 1:2 and 1:4, respectively (Tables 4.3 & 4.4). This is a direct consequence of heterotopic microorganisms including DPAOs due to higher heterotopic oxygen uptake. As DPAOs can only partially utilized the NO₂-N due to the presence of ordinary denitrifiers, thus continue to use oxygen as the electron acceptor, thereby, depriving the ammonium oxidizing bacteria from oxygen. As ammonium oxidation was negatively impacted, phosphorus removal was also negatively affected due to the lack of NO₂-N availability and limited DO. However, the effect of low DO is less severe on DPAOs than nitrifiers, as evidenced by the much higher increase in final effluent ammonia concentration relative to phosphorus.

Scenario 2. Varying dissolved oxygen concentration at N-sludge to P-sludge ratio of 1:4

In order to confirm the competition for oxygen between DPAOs and AOB, the oxygen concentration was further reduced to 0.2 mg/L at N-sludge to P-sludge ratio of 1:4. Table 4.5 shows the initial, end of anaerobic phase and final effluent concentration.

Table 4.5 N, P, C transformation at N-sludge to P-sludge ratio 1:4 and dissolved oxygen concentration 0.2 mg/l

Concentration (mg/L)	Initial	Anaerobic Effluent	Final Effluent
NH ₄ -N	25	22.8	19.6
PO ₄ -P	3	23.5	9.59
sCOD	100	21	14
NO ₃ -N	0	0	0.6
NO ₂ -N	0	0	0.07

As seen in Table 4.5, under severe DO limited situation ACR was further reduced to 22%. However, phosphorus continued to uptake by the DPAOs even in the absence NO₂-N. This result showed that denitrifying phosphorus accumulating organism can be detrimental for nitrifiers in DO limited condition in activated sludge process in the absence of abundant nitrite for P-uptake.

4. Conclusion

This study addressed the competition between denitrifying phosphorus accumulating microorganism and nitrifiers for dissolved oxygen in biological nutrient removal process. The enriched DPAOs culture, capable of using both nitrite and oxygen as electron acceptors, tends to dominate oxygen consumption under DO limited condition in the absence of nitrite. This can significantly impact the overall nutrient removal for EBPR processes operated at low DO concentration.

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Chapter 5

Partial Nitrification-Denitrifying Phosphorus Removal (PNDPR) For Energy and Carbon Minimization

1. Introduction

Nutrients in wastewater effluents, i.e., nitrogen (N) and phosphorus (P), have elicited significant interest because of eutrophication of lakes in different parts of the world. According to the USEPA, nearly 25% of the water body impairments are caused by nutrient-related issues (USEPA, 2007). In order to minimize the extent of nutrient impairments from point sources, stricter jurisdictional regulations for N & P discharges have been imposed.

In traditional biological nitrogen removal process, ammonium ($\text{NH}_4\text{-N}$) is completely oxidized to nitrate ($\text{NH}_4\text{-N}$ to $\text{NO}_2\text{-N}$ by ammonium oxidizing bacteria (AOB) and $\text{NO}_2\text{-N}$ to $\text{NO}_3\text{-N}$ by nitrite oxidizing bacteria (NOB)) and subsequently denitrified to N_2 gas by ordinary heterotrophic microorganisms using organic carbon as electron donor (Metcalf and Eddy, 2014). This process is usually challenging for carbon limited wastewater, where organic carbon is limited for complete nitrogen removal. Since nitrite is an intermediate compound in both nitrification and denitrification process, stopping nitrification at nitrite and subsequently denitrifying from nitrite will achieve many benefits including: (1) 25% reduction in aeration, (2) 40% reduction in carbon requirement, (3) significant reduction in biomass production (Peng & Zhu, 2006).

EBPR is an environmentally friendly-sustainable wastewater treatment process that can maintain low effluent phosphorus concentration. Fundamentally, EBPR consists of an alternating anaerobic-aerobic operational sequence that promotes the growth of PAOs, which can store VFAs under anaerobic conditions as polyhydroxyalkanoate (PHA) and utilize them under aerobic conditions along with phosphorus uptake (Adrian Oehmen et al., 2007).

As an alternative to a traditional EBPR (A/O) process, Kuba et al. (1996) proposed an anaerobic-anoxic (A_2) process which relies on the denitrifying capability of PAOs. The process is particularly beneficial for low COD wastewater, as the same PHA can be utilized for both denitrification and P-removal. Besides, the A_2 process can significantly reduce aeration and sludge production. For A_2 process, although phosphorus is taken anoxically, usually nitrification is required for providing the $\text{NO}_x\text{-N}$ for denitrification. This can either be done in a single sludge or two sludge system. In a single sludge system, prolonged aeration can be detrimental for denitrifying PAOs

(DPAOs) as a significant fraction of intracellular PHA is oxidized by DPAOs aerobically reducing (Chung et al., 2006; Kuba et al., 1996) PHA available for simultaneous denitrification and P-removal. In a two-sludge configuration, the wastewater is typically fed to an anaerobic reactor where most of the readily biodegradable carbon, including, VFAs are taken up by DPAOs and stored as intracellular PHA. The mixed liquor from the anaerobic reactor is settled in a clarifier where the ammonia and phosphorus enriched supernatant is sent to an aerobic reactor for nitrification. The anaerobic sludge and nitrified stream are then sent to an anoxic tank where simultaneous denitrification and phosphorus removal takes place. A second settler is required for separating the denitrifying sludge from treated water and send them back to the anaerobic reactor (Kuba et al., 1996; Zhou et al., 2008). For the two sludge process, the COD consumption is 50% less than conventional A/O process and the oxygen requirement and sludge production decrease by about 30% and 50%, respectively (Kuba et al., 1996). However, the bottle-neck problem of the two-sludge process is high effluent ammonia concentration since a significant proportion of influent ammonia is transferred to the anoxic tank via the anaerobic sludge. This problem is usually minimized by maintaining a high-volume exchange ratio for the nitrification tank which requires excellent settleability of the anaerobic sludge.

Since COD is limiting substance in wastewater and aeration is the most energy-intensive operation in municipal wastewater treatment plant, COD and aeration-energy optimization has been a topic of the recent research subject (Li et al., 2019; Roots et al., 2020; Wang et al., 2015; Yang et al., 2016) Several studies have reported that nitrification-denitrification can occur simultaneously at low DO conditions, which is more commonly known as simultaneous nitrification-denitrification (SND) (Bertanza, 1997; Helmer & Kunst, 1998; Keller et al., 1997; Münch et al., 1996).

Many studies have reported simultaneous denitrification and phosphorus removal by DPAOs, which are primarily based on two sludge process originally proposed by Kuba et al., 1996 (Bernet et al., 2000; Zhou et al., 2008). One of the major drawbacks of this process is when partial nitrification is used for the high ammonia wastewater, the resulting high nitrite concentration in the anaerobic-anoxic (A_2) reactors can significantly inhibit DPAO activity (Meinhold et al., 1999). This problem can be potentially overcome

by designing a SNDPR process based on anaerobic-aerobic configuration to minimize nitrite concentrations. When denitrification is primarily carried out by DPAOs, this can significantly reduce overall carbon consumption for BNR since the same intracellular PHA will be used for both denitrification and phosphorus removal. This process can further reduce sludge production by 20%-30% , since DPAOs are 40% less efficient in generating energy compared to PAOs (Murnleitner et al.,1997). In addition, if SND via nitrite is attempted at low DO conditions, this can significantly reduce the aeration requirement in BNR. The majority of the SNDPR studies reported in the literature operate at moderate to high DO (1 mg/L and above) and high COD/N ratio (6 to 20) (Li et al., 2019; Roots et al., 2020; Wang et al., 2015; Yang et al., 2016). These high COD/N ratios are difficult to attain in municipal wastewater without carbon supplementation. However, carbon supplementation is an additional cost for wastewater treatment plants and compromises the true benefit of SNDPR.

Moderate to high DO concentration is usually found to be unfavorable for the enrichment of DPAOs due to a lack of expression of nitrite reductase enzyme in *Accumulibacter* PAOs. Microbial communities at moderate to high DO were found to be dominated by *Candidatus Accumulibacter* PAOs (50%-70%), *Competibacter* and *propionivibrio* GAOs (15%-25%), and *Candidatus Accumulibacter* DPAOs (15%-25%)(Roots et al., 2020; X. Wang et al., 2015). Therefore, high DO anaerobic-aerobic operations are more favorable for combined SND and aerobic P-removal instead of simultaneous nitrification-denitrifying P-removal(Roots et al., 2020; Q. Yang et al., 2016). Also, the presence of GAOs usually requires a higher COD/N ratio for denitrifying P-removal, as evidenced in the literature (Bassin et al., 2011; He et al., 2016; Li et al., 2019). The aforementioned studies, using synthetic wastewater (SRT 15-25 days and HRT 12-16 hr), reported that at DO concentrations of 1-4 mg/L, and COD/N ratios of 6-10, P-removal via DPAOs accounted for 30%-50% of the total P-removed. The lower anoxic P-removal was primarily due to the competition from GAOs and high DO operation.

This study aims at developing a BNR system using anaerobic-aerobic SBR integrating partial nitrification-denitrifying P-removal for carbon and energy-efficient N and P removal. The unique feature of the SBR was very low DO (0.3 ± 0.05 mg/L) and

low COD/N ratio (4 mg COD/mg N). Several batch studies were conducted to elucidate the pathways for N and P-removal. This study also investigated the relative abundance of various microorganisms and their role in the PNDPR system.

2. Materials and Methods

2.1. DPAO enrichment sequencing batch reactor

DPAOs were enriched in a 2L sequencing batch reactor according to the method outlined in the literature (Dai et al., 2017). The reactor was operated for 3 cycles/day with a volume exchange ratio of 50%. Synthetic wastewater was used for DPAOs enrichment in the mother reactor. The operational sequences were as follows: filling (10 min), anaerobic (90 min), anoxic (180 min), aerobic (120 min), settling (70 min), and decanting (10 min). During the anoxic period, $\text{NO}_2\text{-N}$ was added from a stock NaNO_2 solution using a peristaltic pump. A total of 15 mg/L $\text{NO}_2\text{-N}$ were added (over 3 equal spikes) in each cycle for the anoxic phosphorus uptake. Inline cyclic tests were performed by collecting samples at regular intervals during a typical operation cycle.

2.2. PNDPR sequencing batch reactor

The experimental study was conducted using a sequencing batch reactor with a working volume of 2 L, fitted with diffused aeration system. Before inoculating with DPAO seed sludge from the mother reactor, the PNDPR-SBR was operated at anoxic-aerobic operational sequence (DO: 0.3 ± 0.05 mg/L, 11 days SRT) to achieve partial nitrification. Once partial nitrification was achieved (nitrite accumulation ratio ~80%), PNDPR-SBR was inoculated with 1:1 (VSS mass basis) DPAO sludge to nitrifying sludge and operated at a DO concentration of 0.3 ± 0.05 mg/L and 15 days SRT. The reactor was operated at a fill ratio of 50% in every cycle with 3 cycles/ day with the following operation sequence: 10 min fill, 90 min anaerobic, 300 min aerobic, 70 min settling, 10 min decanting. Room temperature was maintained between 23-25°C. The dissolved oxygen concentration during the aerobic phase was controlled using a PLC based DO controller. The air flow was controlled at 0.1 litre/min and the DO controller supplied air intermittently using on/off control sequence to maintain the DO level between 0.25-0.35 mg/L. The pH of the system was observed to be between 7.5 and 8.1

without active control. After settling phase, 1 L supernatant was decanted and 1L fresh feed was charged into the reactor. Sludge wasting was done once a day at the end of the aerobic cycle after accounting for the effluent VSS to maintain a solids retention time (SRT) of 15 days.

2.3. Wastewater and seeding sludge

Synthetic wastewater was used throughout the operational period in this study. The mother DPAOs reactor was fed synthetic wastewater with the following characteristics: 160 mg/L COD (acetate), 8 mg/L $\text{NH}_4\text{-N}$, 6 mg/L $\text{PO}_4\text{-P}$, and trace metals (mg/L): MgSO_4 (69.6), $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (0.06), $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ (0.24), $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ (0.24), and ZnCl_2 (0.3). In order to prevent nitrification during the aerobic phase 50 mg/L allylthiourea was also added in each phase. PNDPR-SBR was fed with the following synthetic influent characteristics: COD (acetate) 180 mg/L, $\text{PO}_4\text{-P}$ 5 mg/L, $\text{NH}_4\text{-N}$ 45 mg/L, alkalinity (as CaCO_3) 280 mg/L, and trace metals (same as mother DPAOs reactor). Both reactors were initially inoculated with nitrifying activated sludge (initial reactor VSS 2 g/L) obtained from the Greenway wastewater treatment plant, London, ON, Canada. Samples from both reactors were collected twice a week and filtered immediately through 0.45 μm filter paper for water quality analysis.

2.4. Analytical Methods

Analytical grade chemicals from Sigma-Aldrich were used throughout the study. The wastewater and mixed liquor were stored at 4°C before prior to analysis. Total suspended solids (TSS), volatile suspended solids (VSS), and alkalinity were quantified standard method APHA 2540D, 2540E, and 2320B, respectively. Following HACH test kits were used for measurement of water quality parameters: total nitrogen (HACH10072), COD (HACH 8000), total phosphorus (HACH 10127), reactive phosphorus (HACH 8114), ammonia (HACH10031), nitrate (HACH 10020), nitrite (HACH 10019), and VFA (ACH TNT 872) HACH TNT 872). All the samples were filtered through 0.45 μm filter paper prior to analysis.

2.5. Simultaneous nitrification-denitrification (SND) efficiency

SND efficiency is defined (Eq. 1) as the loss of nitrogen in a typical operation cycle after accounting for biomass synthesis:

$$\%SND = [(NH_{4,i} - NH_{4,e} - NO_{2,e} - NO_{3,e} - N_{syn}) \div (NH_{4,i} - N_{syn})] \times 100\% \quad (Eq.5.1)$$

Where, $NH_{4,i}$ is the influent ammonia-N concentration (mg/L), $NH_{4,e}$ is the effluent ammonia -N concentration(mg/L), $NO_{2,e}$ is the effluent nitrite-N concentration(mg/L), $NO_{3,e}$ is the effluent nitrate -N concentration(mg/L), N_{syn} is the nitrogen used (mg/L) for biomass synthesis by ordinary heterotrophs.

2.6. Inline and batch cyclic studies

The PNDPR reactor reached quasi-steady-state approximately within 70 days of start-up. Two inline cyclic tests were performed to confirm that simultaneous nitrification-denitrifying phosphorus removal was sustained steadily in the PNDPR reactor. The cyclic tests were found to be reproducible within a 7 days operational period. Liquid phase concentrations of NH_4 -N, NO_3 -N, NO_2 -N, PO_4 -P, and sCOD were measured at specific time intervals. Furthermore, the following batch tests were also performed once for each to analyze the pathways for denitrification and phosphorus removal in the PNDPR system:

2.6.1. Evaluation of nitrite /nitrate accumulation in the PNDPR reactor at low DO without COD addition

Since DPAOs can perform denitrification using both nitrate and nitrite, this test was performed to investigate whether PNDPR was achieved via the nitrite or nitrate pathways. 250 mL of mixed liquor was collected from the PNDPR reactor at the beginning of the anaerobic cycle prior to COD addition. The reactor was aerated at a controlled DO of 0.3 ± 0.05 mg/L for 5 hours. Liquid phase concentrations of NO_2 -N, NO_3 -N, and NH_4 -N were measured to determine the extent of nitrite accumulation in the PNDPR reactor.

2.6.2. Evaluation of the nitrite or nitrate reduction potential of the biomass

This test was performed to determine whether nitrite and/or nitrate can be used as electron acceptor by the microbial community in the PNDPR reactor for denitrifying phosphorus removal. 250 mL of mixed liquor was collected from the PNDPR reactor at the end of anaerobic cycle (i.e. after P release) and spiked with concentrated NaNO_3 solution to achieve a $\text{NO}_3\text{-N}$ concentration of 25 mg/L or with concentrated NaNO_2 to a total $\text{NO}_2\text{-N}$ concentration of 22 mg/L (over 4 spikes of 10, 5, 5, 2 mg/L) was added to facilitate denitrifying phosphorus removal. No aeration was provided during these experiments.

2.6.3. Evaluation of aerobic P-uptake alone at low DO condition

This test was conducted to evaluate the aerobic P-uptake kinetics at low DO condition. 250 mL of mixed liquor from the PNDPR reactor were collected at the end of anaerobic period and spiked with allylthiourea to an initial concentration of 100 mg/L to prevent nitrification. The reactor was aerated under controlled DO concentration of 0.2-0.3 mg/L for 5 hours.

2.6.4. Evaluation of denitrifying glycogen accumulating organisms (DGAOs) activity in PNDPR reactor

This test was conducted to investigate any potential DGAOs-DPAOs cooperation for nitrogen and phosphorus removal in the PNDPR reactor as outlined by (Rubio-Rincón et al., 2017). 250 mL of mixed liquor was collected from the PNDPR reactor at the beginning of the anaerobic cycle. The batch reactor was operated for 7 hours including 90 minutes anaerobic and 330 minutes of low DO aerobic period (0.3 ± 0.05 mg/L), similar to the main reactor cycle times. After 90 minute of anaerobic contact time, 25 mg/L of $\text{NO}_3\text{-N}$ was spiked to the reactor for anoxic P-removal. The tests were conducted at 2 different pH settings: (1) Anaerobic (pH 6.2)/Anoxic (pH 7.5), and (2) Anaerobic (pH 7.8)/Anoxic (7.5). The pH was controlled using 0.1(M) NaOH and 0.1(M) HCl.

2.7. Microbial Analysis

Microbial community tests were conducted on biomass at day 152 after the reactor reached steady state. The concentrated samples using centrifugation were sent to Microbe Detectives LLC® for DNA extraction and detection of microbes. The 16S rRNA gene of V4 variable region PCR primers 515/806 were used for detection. A single-step PCR (30 cycle) using the HotStarTaq Plus Master Mix Kit (Qiagen, USA) under the following conditions: 94°C (3 minutes), followed by 30 cycles (5 cycle used on PCR products) of 94°C (30 seconds), 53°C (40 seconds) and 72°C (1 minute), followed by an elongation step at 72°C (5 minutes) was performed. An Ion Torrent PGM was used for sequencing following the manufacturer's guidelines. A proprietary analysis pipeline was used for processing the sequence data. In summary, sequences were depleted of barcodes and primers, then sequences <150bp removed, sequences with ambiguous base calls and with homopolymer runs exceeding 6bp were also removed. Sequences were denoised, Operational taxonomic units (OTUs) generated and chimeras removed. OTUs were defined by clustering at 1% divergence (99% similarity). Finally, taxonomical classification of OTUs were conducted using BLASTn against a database derived from the RDPII (<http://rdp.cme.msu.edu>) and NCBI (www.ncbi.nlm.nih.gov).

3. Results and Discussions

3.1 DPAOs enrichment in mother SBR reactor

During a typical operational cycle in the mother SBR most of the readily biodegradable carbon was utilized for the synthesis of PHA in the anaerobic phase (Appendix B, Fig. S1). The specific phosphorus release rate (SPRR) was found to be 53 mgPO₄-P/g-VSS.hr (Appendix B, Table S1) which is higher than the typical literature reported values of 5-32 mgPO₄-P/g-VSS.hr (Brdjanovic et al.,1998; Kuba et al., 1997; Mamais & Jenkins, 1992; Monti et al.,2007). The literature reported SPRR values are mostly for EBPR sludge acclimatized with municipal wastewater. Kuba et al.,1993 reported that for enriched DPAOs culture (12.6% P-content) maximum anaerobic P-release rate was found to be between 40-60 mgPO₄-P/g-VSS.hr. In the current study, the P-content of the sludge in the mother reactor was found to be 11.5% of VSS by weight. Therefore, the high P-release rate signifies successful enrichment of DPAOs culture in

the mother reactor. The anoxic phases depleted all the added $\text{NO}_2\text{-N}$ along with phosphorus uptake. This confirms that DPAOs were successfully expressed with nitrite reductase enzyme in the mother DPAOs reactor. The specific P-uptake rates ($3.7\text{-}5.3 \text{ mgPO}_4\text{-P/g-VSS.hr}$) in the anoxic and aerobic phases were found to be significantly lower than literature values (Appendix B, Table S1). The reported literature values for specific aerobic P-uptake rate are between $5\text{-}20 \text{ mgPO}_4\text{-P/g-VSS.hr}$ (Brdjanovic et al., 1998; Kuba et al., 1997; Mamais & Jenkins, 1992; Monti et al., 2007). The lack of active pH control during the anoxic phase was primarily responsible for lower P-uptake rate. The pH increases from 7.3 to 8.2 at the end of anoxic cycle also significantly impacted the aerobic P-uptake rate as seen in Table S1 (appendix B). The average nitrogen- reduced to phosphorus-uptake rate ratio was found to be 1.05 which is lower than the typical literature reported values of 1.3-1.5 (Dai et al., 2017; Peng et al., 2011) which signifies 20%-30% lower carbon utilization efficiency in the enriched DPAOs culture.

3.2 Start up and operational performance of the PNDPR system

The entire operation of the PNDPR system was divided into 2 phases aimed at: (1) achievement of partial nitrification at low DO and short SRT, (2) partial nitrification and denitrifying phosphorus removal at moderate SRT.

In Phase 1, the SBR was inoculated with returned activated sludge from Greenway WWTP and operated 3 cycles/day. Low DO ($0.3 \pm 0.05 \text{ mg/L}$) facilitated the washout out of NOB and stable nitrite accumulation ratio upto maximum of 85% (Appendix B, Fig. S2). Insignificant nitrite was observed in the effluent due to high NOB population in the first 2 weeks of operation. At start-up, the apparent specific growth rates (24°C , DO 0.25 mg/L , and SRT 11 days) of AOB and NOB were calculated to be, 0.11 and -0.01 d^{-1} (Appendix B), respectively. The growth differential was favorable for NOB washout and after about 2 weeks, NOB washout started to take place and in 45 days stable nitrite accumulation was achieved with NAR ranging from 80% to 85%. Ammonium conversion ratio (ACR) found to be more than 80% throughout the period of reactor operation. In about 40 days, mixed liquor volatile suspended solid concentration stabilized at 240 mg-VSS/L .

In phase 2, the SBR was inoculated with mixed liquor from mother DPAOs reactor at 1:1 (VSS mass ratio) DPAO-sludge to nitrifying sludge and operated at a DO 0.3 ± 0.05 mg/L and 15 days SRT. From the operational performance of phase 1, at 85% NAR, the SND and phosphorus removal efficiencies at start up were anticipated to be similar since the DPAOs in the mother reactor were already acclimatized with nitrite. However, it can be seen from Fig. 5.1a that both nitrogen and phosphorus removal performances immediately dropped to 46% and 25%, respectively. Between day 1 and day 22, after DPAOs inoculation, effluent $\text{NO}_x\text{-N}$ concentration averaged 10 mg/L (Fig. 5.1b).

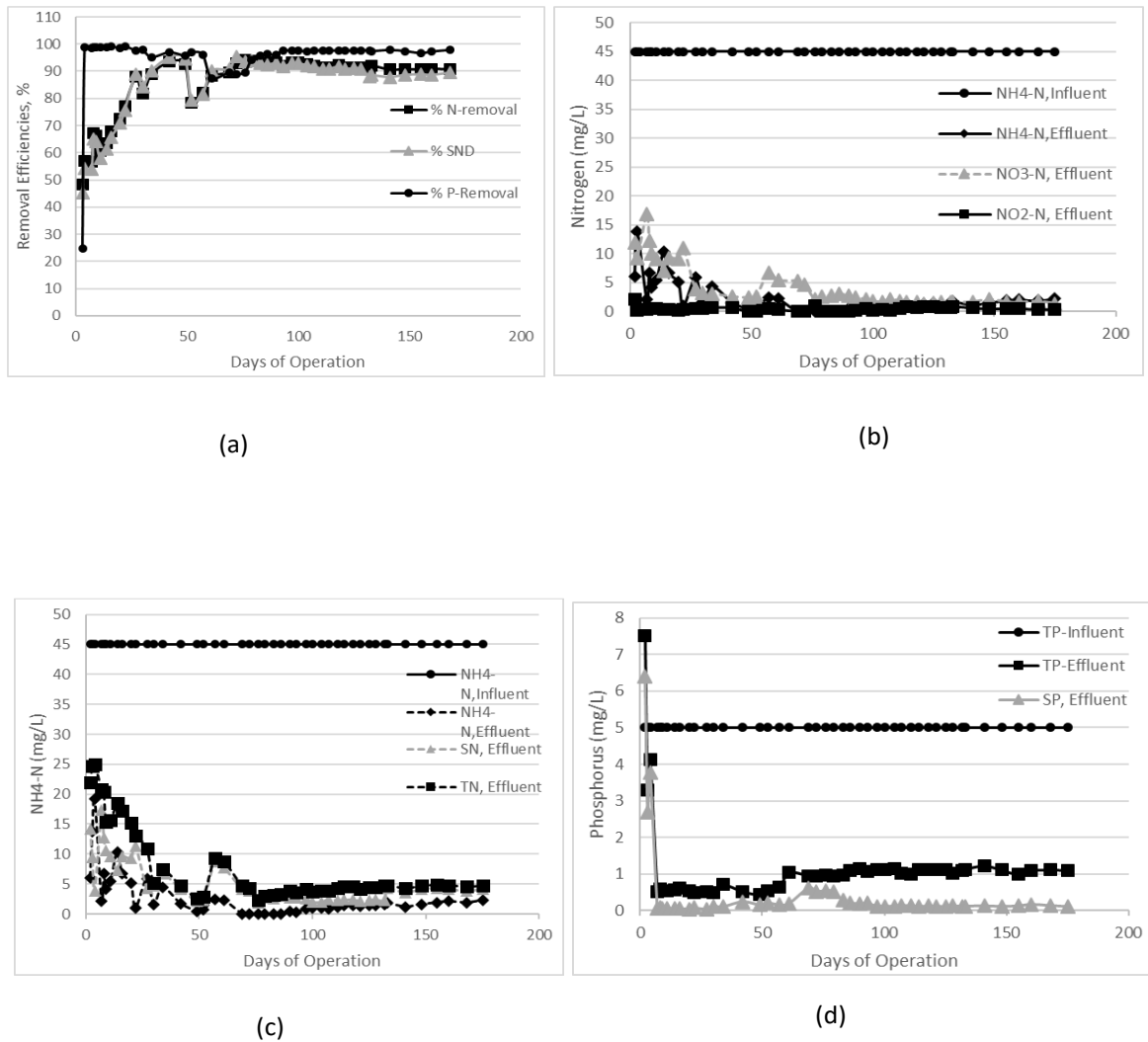


Figure 5.1 Performance of PNDPR system (phase 2) over the 175 days operational period

This could be potentially be due to the carryover of NOBs from the DPAOs mother reactor as the allylthiourea in DPAOs mother reactor did not completely stop the nitrification at the given biomass concentration in the DPAOs mother reactor. Since the DPAOs in the mother reactor were acclimatized to nitrite and high DO (3-4 mg/L), the sudden shift to a low DO (0.3 ± 0.05 mg/L) and nitrate based environment significantly impacted the N,P removal. However, as seen in Fig. 5.1a, phosphorus removal recovered faster than nitrogen removal, implying that DPAOs were able to rapidly acclimatized to low DO in about 7 days . The system reached quasi-steady state in about 4 SRT turnover (Fig.5.1) where N, P removal, and SND percentages reached above 80%. Taking into account the biomass yield of 0.18 g VSS/g COD and P-content of ordinary heterotrophs about 2%, 2 out of 14 mg $\text{PO}_4\text{-P/day}$ was removed by biomass synthesis, indicating that 86% of the influent phosphorus was removed by DPAOs. The SND efficiency in the current study is about 30%-40% higher compared to the literature reported for suspended sludge SNDPR processes operated at low DO (0.5-1 mg/L) and moderate SRT (10-15 days) (Wang et al., 2015). Figs. 5.1c & 5.1d shows that during the period between days 50 and 70 effluent SN and SP concentration increased from 2 to 4 mg/L and 0.22 to 0.62 mg/L, respectively. This was primarily due to a temperature shock in the lab that increased the average lab temperature from 25°C to about 34°C. After day 70, as the lab temperature averaged at 25°C, the steady state concentrations were recovered. The temporary increase of SN, SP could be potentially due the increased biomass decay coefficient at high temperature causing excessive nitrified nitrogen and effluent soluble phosphorus. Throughout the study, effluent total and soluble COD remained as low as 20 and 5 mg/L, respectively. Due to excellent settling characteristics of the flocculant sludge, effluent TSS, VSS remained as low as 7 and 5 mg/L, respectively. The reactor was operated for 175 days and the reactor performance was found to be stable during the entire steady state period (beyond 70 days).

The operational performance in phase 2 suggests that operating EBPR at low DO condition (0.2-0.3 mg/L) integrates biological phosphorus removal with denitrification via DPAOs. In this particular system, both denitrification and phosphorus removal were driven by DPAOs since biodegradable organic carbon (acetate) was completely depleted in the anaerobic phase. The PNDPR system will be particularly advantageous for

wastewaters with limited organic carbon. In addition, a low DO system implies higher oxygen transfer efficiency with a lower aeration energy.

3.3 Nitrogen and Phosphorus mass balance at steady state

The nitrogen balance closed very well with the sum of clarified effluent-N, WAS-N, and denitrified-N accounting for about 98% of the influent total nitrogen. Fig. 5.2a shows the distribution of influent nitrogen across various process streams.

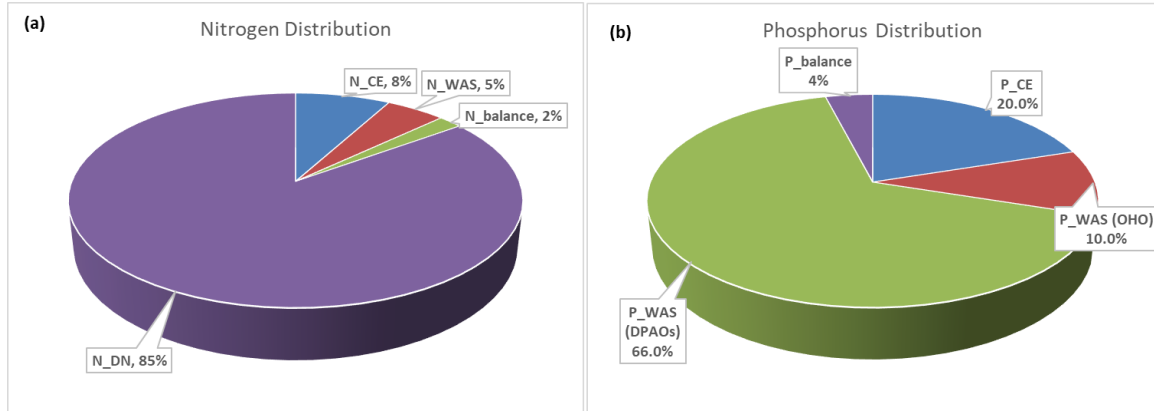


Figure 5.2 Distribution of influent nitrogen (a) and phosphorus (b) across various effluent streams

Approximately 11% of the influent nitrogen ended up in the clarified effluent mostly as $\text{NO}_x\text{-N}$. Also, 85% of the influent nitrogen was denitrified which is in agreement with the high SND efficiency (85% to 90%) of the PNDPR system. Approximately 5% of the influent nitrogen also partitioned in the biomass via cell synthesis and left the system with the activated sludge.

The phosphorus balance also closed very well with the sum of effluent total-P and WAS-P accounting for about 96% of the influent-P. Fig. 5.2b shows the partitioning of the total influent phosphorus into clarified effluent and biomass. The reactor showed a net phosphorus removal efficiency of more than 80% with about 76% of the influent phosphorus in waste activated sludge and 20% in clarified effluent. This clearly implies an active biological phosphorus removal in the reactor. The P-content of the biomass at steady state was about 15% .

3.4 Inline cyclic studies in the PNDPR-SBR system

The kinetics of carbon and nutrient removal were investigated by analyzing a typical operational cycle (8hr) once the reactor reached steady state. Fig. 5.3 shows the variations in sCOD, nitrogen, and phosphorus in the parent SBR in its steady state operation.

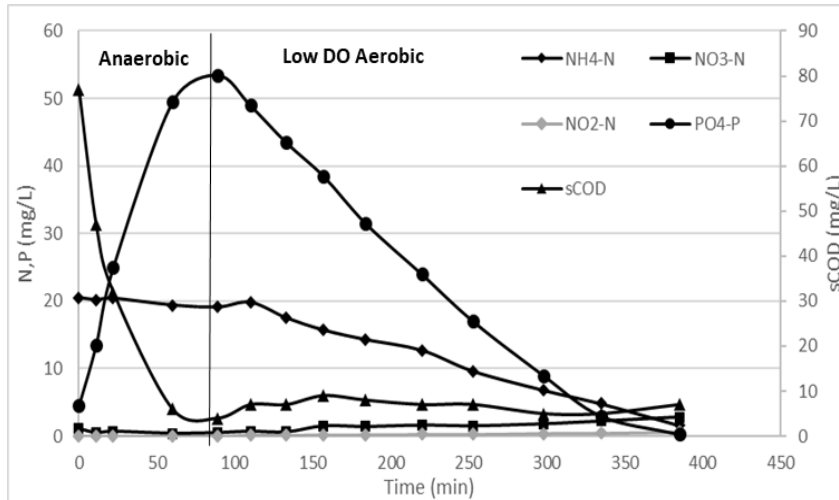


Figure 5.3 Variation of N, P, and sCOD in a typical cycle in PNDPR-SBR operated with synthetic wastewater

The initial sCOD, NH₄-N, NO₃-N, NO₂-N, and phosphorus concentrations during the anaerobic stage were 80, 21, 1, 0.08, and 4.5 mg/L, respectively. During the anaerobic stage, acetate was completely consumed. Poly-P hydrolysis affected an increase in orthophosphate concentration to 54 mg/L. The ratio of P-released to COD utilized was found to be 0.64 which is higher than the typical value of 0.3-0.4 (Kuba et al., 1993, 1997) This signifies a high P-content of the PAO biomass. Inorganic nitrogen concentrations remained almost unchanged during the anaerobic phase. In the subsequent aerobic phase, orthophosphate was taken up along with oxidation of NH₄-N with very little accumulation of nitrate/nitrite signifying the occurrence of simultaneous nitrification and denitrifying phosphorus removal. During the aerobic cycle, NH₄-N and PO₄-P concentration decreased by 19.1 and 53.6 mg/L with effluent NO₃-N, NO₂-N concentrations of 2.5 and 0.5 mg/L, respectively signifying a loss of 16 mg/L nitrogen. Since almost no sCOD remained after the anaerobic stage for exogenous denitrification by OHO, denitrification during the aerobic stage can be entirely contributed to DPAOs using endogenous carbon.

Nutrient removal kinetics and performances were also calculated from the cyclic test (Appendix B, Table S2). The biomass-specific P-release rate was found to be 53 mg/g-VSS.hr which is higher than the typical value 5-32 mg/g-VSS.hr (Mamais and Jenkins, 1992; Kuba et al., 1997; Brdjanovic et al., 1998; Monti et al., 2007). Biomass-specific P-uptake rate was found to be 21 mg/g-VSS.hr which is consistent with the reported literature values of 6-21 mg/g-VSS.hr (Kuba et al., 1997; D. Mamais & Jenkins, 1992). Biomass-specific ammonium uptake rate was found to be 4.7 mg/g-VSS.hr which is reasonable considering a low DO operation. SND, P-removal, and N-removal efficiencies were found to be 80%, 93%, and 77%, respectively.

3.5. Batch studies for evaluation of N and P removal pathways

Since DPAOs can utilize nitrate and nitrite under anoxic conditions and oxygen under aerobic condition in the absence of $\text{NO}_x\text{-N}$, it is important to evaluate their relative contribution in the PNDPR system. Batch studies 1, 2, and 3 were conducted in order to find the dominant electron acceptor in the PNDPR system while batch study 4 was conducted in order to find any contribution of GAOs in the denitrifying phosphorus removal in the PNDPR system.

3.5.1. Batch study 1: Nitrite accumulation at low DO condition without COD addition

A batch study was conducted to investigate the major nitrification product in the PNDPR reactor operated at low DO condition (batch study#1). The biomass was subjected to low DO (0.2-0.3 mg/L) without COD addition in the absence of any anaerobic period. Ammonium was completely oxidized (AUR 8.5 mg/g-VSS.hr) to nitrite and nitrate with a nitrite accumulation ratio of 82% (Fig.5.4a).

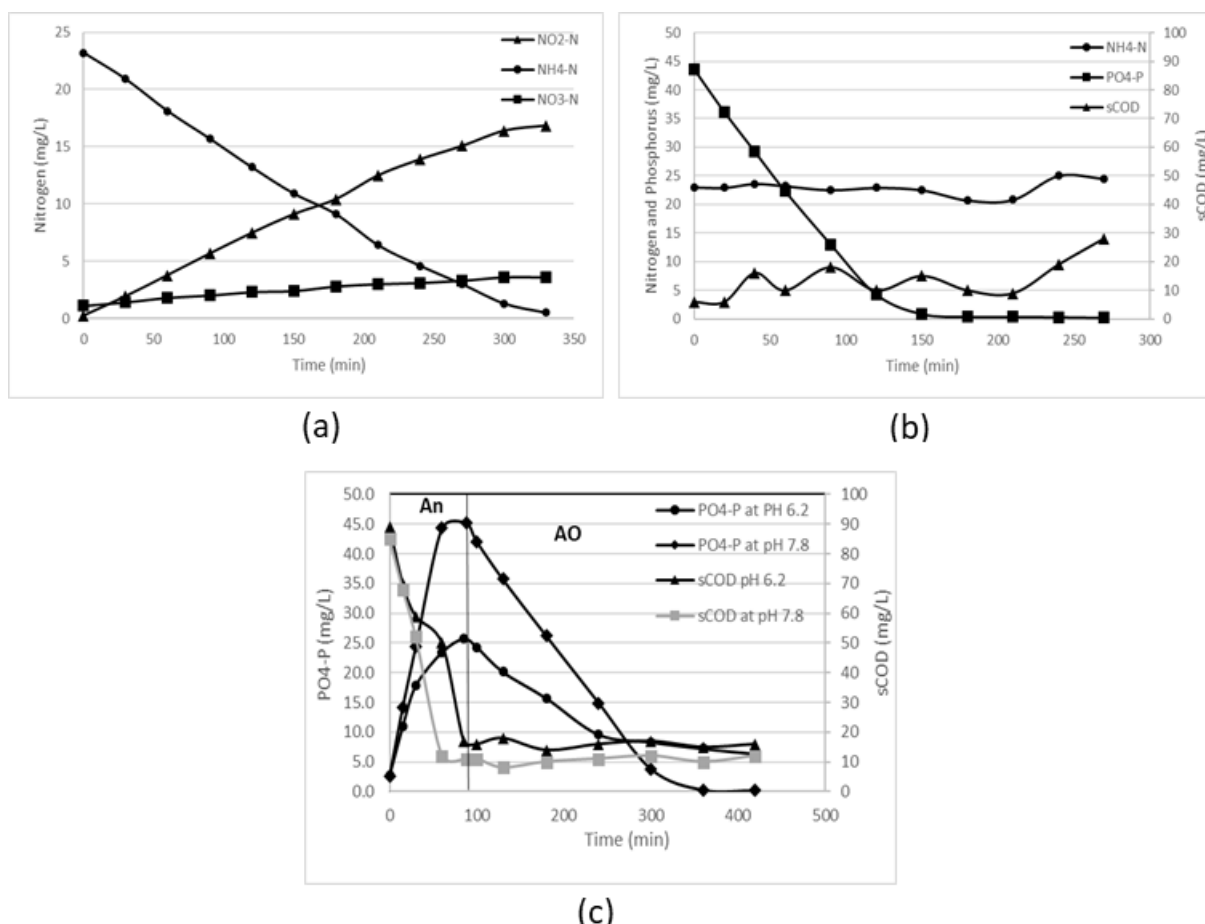


Figure 5.4 Evaluation of N & P removal pathways: (a) nitrification at low DO without COD addition, (b) aerobic P-uptake by DPAOs at low DO, (c) anoxic phosphorus uptake profile at pH 7.5 with anaerobic phosphorus release at different pH of 6.2 & 7.8

This clearly indicates that nitrite instead of nitrate was the major nitrification product in PNDPR system and denitrifying phosphorus removal took place via nitrite pathway. In addition, the biomass was still able to achieve approximately 12% SND indicating PHA left over from the previous cycle were used for denitrification.

3.5.2. Batch study 2: Comparison of phosphorus uptake using nitrate versus nitrite as electron acceptors

The comparison of phosphorus removal with nitrate and nitrite as electron acceptor is summarized in Table 5.1. There is no significant difference in COD removal as majority of the COD was removed during the anaerobic stage.

Table 5.1 Stoichiometry and kinetics of DPAOs using nitrate or nitrite as an electron acceptor

Electron acceptor	COD removal,%	N-removal ,%	P-Removal,%	Average P-uptake rate (mg-P/g-VSS.hr)	Average N-reduction rate (mg-N/g-VSS.hr)	$\Delta P/\Delta N$	$\Delta COD/\Delta N$	Carbon saving for SNDPR, %
Nitrate	94	38	99	14	3.6	3.9	8.5	-
Nitrite	95	85	99	13	7.3	1.8	4	53%

Nitrogen removal efficiency was significantly higher (85%) with nitrite as compared to nitrate (38%). Sp. denitrification rates (SDNR) for nitrite and nitrate were found to be 7.3 ,and 3.6 mg-N/g-VSS.hr, respectively. For pre-anoxic zone treating domestic wastewater, depending on the type and amount of readily biodegradable carbon, SDNR may range from 1.7-17.5 mg NO₃-N/ g-VSS.hr, and 3- 27 mg NO₂-N/ g-VSS.hr (Lee & Yun, 2014, Metcalf & Eddy, 2014, Peng & Zhu,2006). For post anoxic denitrification where substrate for denitrification is provided by endogenous decay, SDNR may vary from 0.63-2.5 mg NO₃-N / g-VSS.hr, 0.9-4 mg NO₂-N / g-VSS.hr (USEPA, 2007; Metcalf and Eddy, 2014, Yan et al., 2019). In a typical anaerobic-low DO aerobic process, denitrification via ordinary denitrifiers is comparable to post anoxic process since majority of the biodegradable carbon is utilized in the anaerobic zone. Comparing the abovementioned post anoxic denitrification rates, DPAOs will outperform ordinary denitrifiers in the absence of readily biodegradable exogenous carbon and contribute to simultaneous N and P removal. Comparing the nitrate versus nitrite reduction rates, nitrite denitrification via DPAOs is significantly faster (7.3 mg NO₂-N / g-VSS.hr) than post anoxic nitrite denitrification (0.9-4 mg NO₂-N / g-VSS.hr) by ordinary denitrifiers. Moreover, carbon demand for denitrification is 53% less via nitrite pathway (Table 5.1). This clearly signifies the benefit of nitrite pathway over nitrate both in terms of DPAOs outcompeting ordinary denitrifiers and carbon savings. In this study, sp. P-uptake rate/ sp. denitrification rate for nitrite and nitrate were 1.8, and 3.9, respectively. The reported typical values for sp. P-uptake rate/SDNR for DPAOs are 1 to

2 and 4 to 6 for nitrite and nitrate, respectively (H. Lee & Yun, 2014; Y. Z. Peng et al., 2011). Ratio of COD utilized to nitrogen reduced was 4 with nitrite and 8.5 with nitrate as electron acceptor. The batch study clearly showed that DPAOs acclimatized at low DO are capable of using both nitrate and nitrite as electron acceptors; albeit, with a reduced efficiency for nitrate. The stoichiometric information from this batch study clearly shows that there will be a significant (~53%) carbon savings when nitrite is utilized as electron acceptor compared to nitrate in simultaneous nitrogen/phosphorus EBPR systems. Thus, achieving nitrite shunt is particularly important for treating carbon limited wastewater for simultaneous N and P removal.

3.5.3. Batch study 3: Kinetics of aerobic P-uptake at low DO condition using oxygen as electron acceptor

Carvalho et al. (2014) reported that aerobic P-uptake rate for Accumulibacter PAOs decreases by about 20% at a DO level of 0.6 mg/L compared to DO 4 mg/L. P-uptake rate decreased in the DO range of 0.1 to 0.6 mg/L by about 70% compared to a DO of 4 mg/L (Carvalho et al., 2014). However, the impact of low DO on DPAOs for aerobic P-uptake has not been reported. Fig. 5.4b shows the aerobic P-uptake kinetics of DPAOs culture at a DO level of 0.3 ± 0.05 mg/L. The sp. P-uptake rate was found to be 19 mg/g.VSS.hr which is within the typical values (6-21 mg/g.VSS.hr) for aerobic P-uptake rate by Accumulibacter PAOs at high DO condition. The high sp. P-uptake rate at low DO reflects non-Accumulibacter DPAOs dominance in the PNDPR reactor.

3.5.4. Batch study 4: Anoxic P-uptake at different pH conditions: role of DGAOs in PNDPR reactor

In order to investigate the role of DGAOs (if any) on the denitrifying phosphorus removal in the PNDPR reactor, two batch studies were conducted at different pH scenarios: (1) Anaerobic (pH 6.2)/Anoxic (pH 7.5), and (2) Anaerobic (pH 7.8)/Anoxic (7.5). According to Filipe et al. (2001), at or above pH of 7.25, PAOs uptake acetate faster than GAOs. Therefore, if the DPAOs are not capable of nitrate reduction and rely on DGAOs for nitrate to nitrite conversion, a lower anoxic P-uptake will occur when the anaerobic pH is maintained at 7.8 and vice versa when the anaerobic pH is maintained

at 6.2. This is due to the fact that at high anaerobic pH (7.8) DPAOs will accumulate VFAs faster, resulting in less carbon available for DGAOs. Similarly, at low pH, DGAOs will have a competitive advantage for carbon storage, perform higher nitrate to nitrite reduction, and facilitate P-uptake by DPAOs (Rubio-Rincón et al., 2017). Fig. 54c shows the P-release and P-uptake characteristics of biomass at different pH. It can be observed from Fig.5.4c that at both pH, acetate was completely consumed. The P-release rate was significantly lower at pH 6.2 (22 mg-P/g-VSS.hr) compared to the pH 7.8 (48 mg-P/g-VSS.hr). However, this does not imply a DGAOs-DPAOs competition for carbon because the P-uptake rate for anaerobic pH 7.8 (13 mg/g-VSS.hr) is significantly higher than anaerobic pH 6.2 (7 mg/g-VSS.hr). In addition, the nitrate reduction was also 2 times higher at pH 7.8 than pH 6.2. Tayà et al. (2013) reported the DGAO culture could not denitrify nitrite, and Zeng et al. (2003) also reported $\text{NO}_3\text{-N}$ reduction is much faster than $\text{NO}_2\text{-N}$ reduction for DGAOs, hence DPAOs get a kinetic advantage over DGAOs for utilizing nitrite. However, in our system at pH 6.2 both nitrate and phosphorus reduction were impacted. If DGAOs were present, a decrease P-reduction but not $\text{NO}_3\text{-N}$ reduction at low pH would have been observed.

Therefore, it is highly likely that DGAOs have been washed out from the PNDPR reactor due to partial nitrification at high NAR (82%). Furthermore, as discussed in section 3.7, most commonly found DGAOs species, such as *Competibacter phosphatis* was not detected and *Deftuvicoccus* & *Propionivibrio* accounted for less than 0.1% in the microbial analysis. This further confirms that the DPAOs in the PNDPR reactor are capable of denitrifying directly from nitrate (without denitrification by DGAOs) in addition to nitrite. The lower P-release/acetate uptake at pH 6.2 is primarily due to lack of hydrolysis of poly-p since less energy is required for acetate transportation at low pH (Smolders et al., 1995). Therefore, a low pH anaerobic condition can impact the P-release, synthesis of PHA, and subsequent anoxic P-uptake by DPAOs due to lack of PHA as evidenced in this study.

3.6 Contribution of nitrifiers, DPAOs, and various electron acceptors to overall nutrient removal

The batch studies clearly showed that ammonium oxidizing bacteria and denitrifying PAOs were the dominant microorganisms for N and P removal in this study. Table 5.2 shows the key microbes and N-P species and their contribution to overall nutrient removal.

Table 5.2 Contribution of nitrifiers, DPAOs, and various electron acceptors to overall nutrient removal performance (daily basis)^a

N and P species	Contribution on nutrient removal (mg)
Net anaerobic PO ₄ -P release	249
Influent PO ₄ -P	15
P-removed by OHO	2
Net (anoxic+aerobic) P-uptake by DPAOs	262
Ratio of P-uptake / P-release	1.06
P-Removal by NO ₂ -N	182
P-Removal by NO ₃ -N	59
P-Removal by O ₂	21
Influent NH ₄ -N	135
N-removed by biomass synthesis	8
NO ₂ -N reduced by DPAOs	101
NO ₃ -N reduced by DPAOs	15
Ratio of PO ₄ -P removal / NO _x -N reduced via DPAOs	2.08
Ratio of DPR via nitrite/DPR via nitrate	3.1

^aCalculations are shown in Appendix B

On a daily basis, 124 mg NH₄-N was oxidized by AOBs and 8 mg NH₄-N was assimilated via biomass synthesis. 22 mg of NO₂-N was further oxidized into NO₃-N by the NOBs corresponding to nitrite accumulation ratio of 82% (Fig. 5.4a). Since all the biodegradable carbon was completely consumed in the anaerobic phase (Fig. 5.4c), NO_x-N reduction was primarily carried out by the denitrifying PAOs along with P-removal. 101 mg NO₂-N and 15 mg NO₃-N was denitrified to N₂ gas by the DPAOs. According to Table 5.2, this corresponds to an anoxic P-removal of 241 mg PO₄-P. Approximately, 2 mg PO₄-P was also removed by ordinary heterotrophs for biomass synthesis. Considering PO₄-P concentration of 44 mg/L at the end of the anaerobic period (Figs.5.4b & 5.4c), 264 mg PO₄-P was removed daily during the low DO aerobic phases implying a P-

uptake/P-release ratio of 1.06 which is well within the reported literature value of 1.05 to 1.10 (Pan et al., 2017; Wang et al., 2015) Therefore, 21 mg PO_4P was removed aerobically using O_2 as electron acceptor which represents 8% of the total daily P-removal. Therefore, of the P removed by EBPR, P-removal percentages via nitrite, nitrate, and oxygen were 69%, 23%, and 8%, respectively. This confirms that phosphorus was primarily removed via the nitrite pathway in the PNDPR reactor.

3.7 Microbial Community Analysis

Many studies have been reported in the literature that operated EBPR at a very low DO concentration with varying phosphorus removal efficiencies (60%-90%) (Li & Chen, 2011; Li et al., 2008; Zheng et al., 2009). However, none of these studies investigated the long term impact of low DO on the microbial community structure. Therefore, it is still unclear how very low DO can impact microbial community structure in EBPR. For conventional EBPR process, a DO concentration of 2-3 mg/L is generally recommended for optimal phosphorus removal (Mulkerrins et al., 2004; Shehab et al., 1996) where *Accumulibacter* is found to be the dominant PAOs in EBPR. However, the fate of *Accumulibacter* is rather unknown at very low DO condition (<0.3 mg/L). Since the operating condition in low DO PNDPR system is not typical, microorganisms with low oxygen half-saturation concentration can potentially survive in such system.

In order to investigate the microbial community of low DO PNDPR system, PCR analysis was performed on the biomass. Fig.5.5 shows the major microbial species found in the bacterial consortium at low DO.

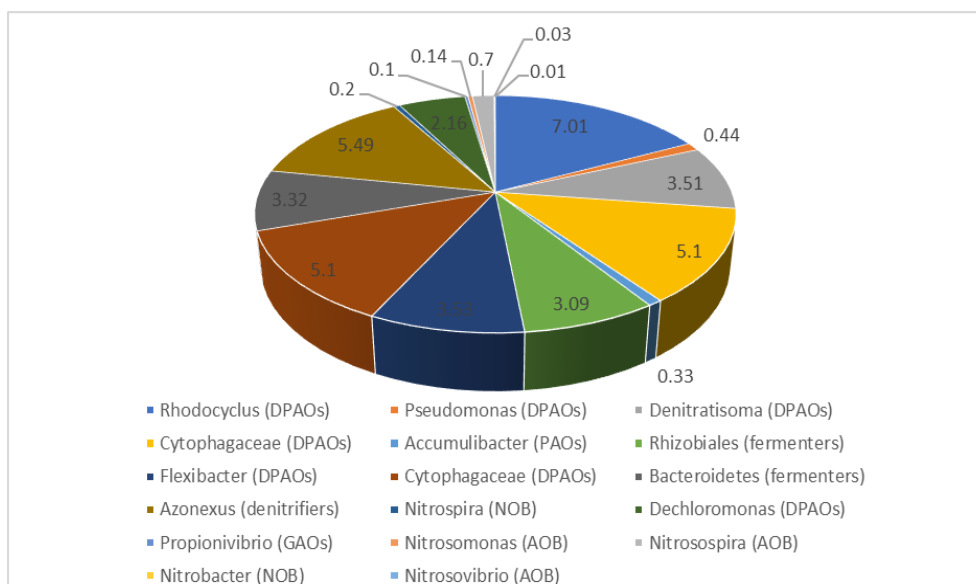


Figure 5.5 Relative abundance of major microbial species in the low DO PNDPR-SBR

The percentage of GAOs (*Propionivibrio*) was found to be insignificant compared to the PAOs, representing less than 1% of the PAO population. Previous studies showed that PAOs have competitive advantage over GAOs at low DO condition, as PAOs have a higher oxygen affinity and thus maintain their activity at low DO concentration, while GAO activity decreased (Carvalho et al., 2014; Lemaire et al., 2006). Compared to DO concentration of 8 mg/L, at DO concentration of 0.6 mg/L, P-uptake rate and PHA consumption rate of PAOs decreased by 20 and 27% , respectively, while the PHA consumption and glycogen production rates of GAOs decreased by 77% and 88%, respectively (Carvalho et al., 2014). Therefore, low DO condition in the current study is highly favorable for GAO washout from the microbial consortium.

Among the phosphorus accumulating organisms, *Rhodocyclus* and *Dechloromonas* spp. from the *Rhodocyclaceae* family and *Cytophaga* from *Cytophagaceae* family were found to be the only PAO microorganisms in the bacterial consortium and represent about 14% of the microbial community. Surprisingly, *Accumulibacter* which is more commonly found in conventional EBPR processes was less than 2% of the overall PAO population. Carvalho et al. (2014) reported that the aerobic metabolic rates (P-uptake, PHA consumption, and glycogen production) of *accumulibacter* PAOs are stable over a wide range of DO above 2 mg/L; however,

metabolic rates drop below 2 mg DO/L and decreased substantially (by about 70%) in the DO range from 0.1 to 0.6 mg/L. This justifies the washout of *Accumulibacter* PAOs in the current study. The dominant PAO species, *Rhodocyclus* and *Cytophaga* bacteria have been observed in many full scale EBPR plants performing simultaneous denitrification and phosphorus removal (Kong et al., 2004; Park et al., 2002; Terashima et al., 2016; Zilles et al., 2002). These DPAO microorganisms are able to assimilate acetate, propionate, and fermented products from more complex carbon. They are able to take up and accumulate orthophosphate when oxygen, nitrate or nitrite are present as electron acceptors. Therefore, in the current study these bacterial species could potentially use all three electron acceptors depending on their location in the microgranule and perform simultaneous denitrification and phosphorus removal.

Three types of ammonium oxidizing bacteria (AOB) was found in the microbial community including: (1) *Nitrosomonas* (0.15%), (2) *Nitrosovibrio* (0.03%) , and (3) *Nitrospira* (0.70%). Some nitrite oxidizing bacteria (NOB), such as *Nitrobacter* (0.03%) and *Nitrospira* (0.20%) were also observed in the reactor. In this study, AOBs represents about 80% of the nitrifier population. The DO half-saturation concentration of AOBs and NOBs are 0.2-0.4 mg/L and 1.2-1.5 mg/L, respectively (Peng & Zhu, 2006). The low DO condition (0.3 ± 0.05 mg/L) was highly favorable for a significant washout of NOBs due to lower oxygen affinity of NOBs compared to AOBs. In the current study, a low NOB:AOB population ratio of 0.25 was highly favorable for nitrite shunt and denitrifying phosphorus removal was primarily occurred by nitrite pathway.

4. Conclusions

A novel single sludge partial nitrification-denitrification SBR system enriched with denitrifying PAOs (DPAOs) was developed and successfully operated at very low DO condition to simultaneously remove nitrogen and phosphorus from low COD wastewater. Low DO condition and partial nitrification favored the selective washout of DGAOs from the PNDPR system allowing DPAOs to fully utilize all the available biodegradable carbon for simultaneous denitrification and phosphorus removal for carbon limited synthetic wastewater. The key findings from this study as follows:

- Long term operation of EBPR at very low DO condition (0.2-0.3 mg/L) favors the washout of DGAOs and Accumulibacter PAOs.
- The metabolic rates of DPAOs remained high at low DO condition and showed comparable EBPR performance to Accumulibacter PAOs at high DO.
- Washout of DGAOs and predominant N-removal via DPAOs significantly improved the anoxic share of P-removal (nearly double the reported literature values of 30%-50%) to 92%.
- Simultaneous nitrite shunt and denitrifying phosphorus removal was observed in the low DO aerobic phase where SND, P-removal, and N-removal efficiencies were as high as 90%.
- As evidenced in the inline cyclic study, the majority of the readily biodegradable and slowly biodegradable carbon was utilized during the anaerobic stage. Thus, DPAOs were primarily responsible for the denitrification instead of ordinary heterotrophs.
- Batch studies confirmed that the DPAOs enriched culture were capable of utilizing oxygen, nitrate, and nitrite as electron acceptor. However, due to significant washout of the NOBs in the PNDPR system, nitrite was the predominant electron acceptor for the phosphorus removal. Of the total P removed by EBPR, P-removal percentages via nitrite, nitrate, and oxygen were 69%, 23%, and 8%, respectively. Utilizing nitrite instead of nitrate signifies a 53% reduction in carbon requirement for simultaneous denitrification and phosphorus removal. Due to the predominance of nitrites, DGAOs were outcompeted by DPAOs.
- In terms of energy savings, low DO PNDPR operation signifies a greater mass transfer driving force for oxygen transfer which translates into reduced air flow and significant saving in aeration cost

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Chapter 6

Simultaneous Nitrification-Denitrifying Phosphorus Removal (SNDPR) at low DO for treating carbon-limited municipal wastewater

1.Introduction

EBPR is a widely used process for efficient and reliable phosphorus removal from wastewater. Traditional fully nitrifying municipal wastewater treatment plants (MWWTP) are not generally optimized for carbon and energy efficiency. With the increasing concern over MWWTP carbon footprint, development of environmentally sustainable and cost-effective carbon and energy efficient nutrient removal processes is critical (Li *et al.*, 2019; Roots *et al.*, 2020; Wang *et al.*, 2015; Yang *et al.*, 2016).

Marcelino *et al.*(2011) demonstrated simultaneous C, N, P removal in a two-sludge system via nitrite pathway. Synthetic wastewater was fed to a heterotopic SBR (HET-SBR) where P-release rate and COD uptake rate occurred at 60 mgPO₄-P/g-VSS.h and 120 mgVFA-COD/g.VSS.h . After settling, the supernatant was sent to an autotrophic SBR (AUT-SBR) for partial nitrification. The DO concentration in the AUT-SBR was maintained between 1.5 and 2 mg/L with an on/off control. The nitrite enriched supernatant from AUT-SBR was send back to the HET-SBR for anoxic P-removal. While the overall C and P removal were almost complete, N-removal was only 75%. .Such process can be challenging for treating high ammonia wastewater due to carryover of large amounts of nitrite from the AUT-SBR which can significantly inhibit DPAO activity in HET-SBR (Meinhold *et al.*, 1999). In addition, a significant portion of the influent ammonia is leftover in the HET-SBR, causing lower overall nitrogen removal. Another bottle-neck problem of this process is alkalinity limitation. Since the effluent leaves the HET-SBR right after the anoxic phase, only a portion of the produced alkalinity is transferred to the N-reactor and used in the nitrification process. This can be particularly problematic when alkalinity is limited as observed by Marcelino *et al.*(2011). In addition, from a practical perspective, operating a two-sludge process is complicated.

A single sludge simultaneous nitrification-denitrification phosphorus removal (SNDPR) system via nitrite pathway is particularly advantageous over the two-sludge system in terms of : (1) lower alkalinity demand due to utilization of regenerated alkalinity from denitrification, (2) lower nitrite accumulation in the system due to simultaneous denitrification, (3) lower capital investment and operational cost because of lesser unit operations, and (4) lower residual ammonia because of complete aerobic oxidation of all organic-N in the influent wastewater. Recently, simultaneous

nitrification-denitrification and phosphorus removal (SNDPR) has elicited significant attention as a feasible alternative to traditional EBPR process (Bassin *et al.*, 2011; He *et al.*, 2016; Jimenez *et al.*, 2014; Ju *et al.*, 2007; Li *et al.*, 2019; Marcelino *et al.*, 2011; Meyer *et al.*, 2005; Roots *et al.*, 2020; Tsuneda *et al.*, 2006; Wang *et al.*, 2016; Wang *et al.*, 2015; Wu *et al.*, 2015; Yang *et al.*, 2016). The microbial consortium of SNDPR primarily consists of ordinary heterotrophs (OHO), nitrifiers, phosphorus accumulating organisms (PAOs), and glycogen accumulating organisms (GAOs). A subgroup of PAOs, commonly known as denitrifying PAOs (DPAOs) plays a significant role in N and P removal as they can utilize both nitrate and nitrite as electron acceptors. The role of DPAOs in denitrifying phosphorus removal has been well documented in the literature (Bernet *et al.*, 2000; Kuba *et al.*, 1996; Zhou *et al.*, 2008). Since DPAOs can simultaneously remove N and P, integrating SND with denitrifying phosphorus removal in a single sludge system minimizes carbon and oxygen requirements.

Wang *et al.* (2015, 2016) investigated a single sludge SNDPR-SBR at moderate DO (1 mg/L) and COD/N ratio 4. Only 65% TN and 37% TP removal was obtained at such low COD/N. VFA supplementation and/or post-denitrification was required to achieve N and P removal efficiencies of 78% and 94%, respectively. Zheng *et al.* (2009) investigated a low DO (0.45 mg/L) SNDPR process operated at low COD/N ratio of 6. Without carbon supplementation N and P removal were only about 61%, improving to more than 80% and 90% with acetate or fermented waste activated sludge liquid supplementation at COD/N ratio to 15. SNDPR was also investigated with aerobic granular sludge (Bassin *et al.*, 2012; He *et al.*, 2016; Wang *et al.*, 2009; Wang *et al.*, 2018) in single-sludge SBR using synthetic wastewater. Average SND, TN, and TP removal efficiencies were 70%, 85%, and 90%, respectively. The majority of these studies were conducted at high DO (1.8-5 mg/L) and high COD/N ratio (7-11).

Even though SNDPR was extensively studied with synthetic and septic tank wastewater, SNDPR was rarely investigated with municipal wastewater. Jimenez *et al.* (2014) reported a full-scale low DO (0.4 mg/L) AO process operated at COD/N ratio 7-10. The process achieved about 85% of N and P removal, however, denitrification and P-removal were primarily carried out by OHO and aerobic PAOs, respectively. Yang *et al.* (2016) also investigated a full-scale SNDPR operated at 1.4 mg/L DO and sCOD/N

ratio of about 5. The process showed good N-removal (90%), however, P-removal was only 76% with an effluent SP of 1-2 mg/L. Recently, Roots et al.(2020) combined SND and P-removal in an SBR treating primary effluent with COD/N ratio of 8 to 10. Even though the SBR successfully achieved N and P removal percentages of 83% and 81%, respectively, the process failed to achieve simultaneous nitrification-denitrifying phosphorus removal via DPAOs.

Moderate to high DO concentration is unfavorable for the enrichment of DPAOs due to a lack of expression of nitrite reductase enzyme in *Accumulibacter*. Microbial communities at moderate to high DO were found to be dominated by aerobic *Candidatus Accumulibacter* (50%-70%), GAO such as *Competibacter* and *propionivibrio* GAOs (15%-25%), and denitrifying *Candidatus Accumulibacter* (15%-25%) (Roots et al., 2020; Wang et al., 2015). Therefore, high DO anaerobic-aerobic processes are more favorable for combined SND and aerobic P-removal instead of simultaneous nitrification-denitrifying P-removal(Roots et al., 2020; Q. Yang et al., 2016). Also, the presence of GAOs usually requires a higher COD/N ratio for denitrifying P-removal, as evidenced in the literature (Bassin et al., 2011; He et al., 2016; Li et al., 2019). Microbial community at low DO (0.45-1 mg/L) and high COD/N ratio (8 and above) were found to be dominated by GAOs and PAO clade II (Zeng et al., 2003; Zheng et al., 2009). Lemaire et al.(2006) reported that the abundance of DPAOs and GAOs in a low DO (0.35-0.5 mg/L) SNDPR process operated at high COD/N ratio of 10 increased by 70%, and decreased by 50%, respectively, over the 5-month study. , At a DO concentration of 0.6 mg/L, P-uptake rate and PHA consumption rate of PAOs decreased by 20% and 27% , respectively, while the PHA consumption and glycogen production rates of GAOs decreased by 77% and 88%, respectively (Carvalho et al., 2014), relative to DO of 8 mg/L. Therefore, very low DO (< 0.5 mg/L) is highly favourable for washout of GAOs in SNDPR.

In contrast to the aforementioned studies where denitrification was primarily carried out by OHOs and P-removal by aerobic PAOs at DO >>1 mg/L and COD/N of 7-15, this study aims to achieve simultaneous nitrification and denitrifying phosphorus removal via DPAOs. A single-sludge SNDPR system, removing C, N, and P from real municipal wastewater without any carbon supplementation, was demonstrated. The

process was operated with continuous aeration at very low DO condition (0.3 ± 0.05 mg/L) to facilitate anoxic P-removal via DPAOs. The reactor was operated at COD/N ratio as low as 5. Cyclic tests confirmed the occurrence of simultaneous nitrification - denitrification and provided insight into the competition between DPAOs and NOBs for nitrite.

2. Materials and Methods

2.1. Wastewater and seed sludge

Municipal wastewater (primary effluent) was collected from Greenway wastewater treatment plant, London, Ontario. Until day 48 the reactor was feed with the primary effluent as is. From day 49 and onward, the primary effluent was diluted two times and spiked with ammonium chloride (if necessary) to maintain the desired COD/N ratio. DPAO inoculum was collected from an ongoing lab-scale parent DPAO-SBR (Zaman *et al.*, 2019), which successfully enriched DPAOs in the activated sludge and operated for more than 12 months. Activated sludge was also collected from a fully nitrifying lab-scale SBR, which had a stable performance with respect to N and P removal from synthetic wastewater for more than six months.

2.2. Batch activity tests of DPAO inoculum

250 mL mixed liquor was collected from the parent DPAO-SBR near the end of the operational cycle. The mixed liquor was washed with deionized water by centrifuging and decanting for 3 times. The mixed liquor was then resuspended in 125 mL of DI water. 125 mL of synthetic wastewater with following characteristics was fed to the reactor: 150 mg/L COD (acetate), 8 mg/L $\text{NH}_4\text{-N}$, 6 mg/L $\text{PO}_4\text{-P}$, and trace metals (70 mg/L MgSO_4 , 0.06 mg/L $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.24 mg/L $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 0.24 $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 0.3 mg/L ZnCl_3). The reactor was operated similar to the parent DPAO-SBR i.e. r: 90 min anaerobic, 200 min anoxic, 130 min aerobic (DO: 2-3 mg/L). 50 mg/L allylthiourea was also added to prevent nitrification during the aerobic contact period. During the anoxic react period, 3 spikes of 7 mg/L $\text{NO}_2\text{-N}$ (as NaNO_2) each were added. The MLSS and MLVSS were 2490 mg/L, and 1790 mg/L, respectively.

2.3. Analytical methods

APHA methods 2540D, 2540E, and 2320B were used for quantification of total and volatile suspended solids, and alkalinity, respectively. HACH water quality parameter test kit was used for quantification of total and soluble nitrogen, COD, total and soluble phosphorus, ammonia, nitrate, nitrite, and volatile fatty acids. Flocculated and filtered COD (ffCOD) fraction of the wastewater were measured according to the method outlined by Mamais *et al.* (1993): 1mL of 100 g/L zinc sulfate solution was mixed with 100mL wastewater sample and mixed vigorously using a vortex mixer for 1 min. The pH was adjusted to 10.5 using 6(M) NaOH, and the solution was allowed to settle for about an hour, and the clear supernatant was taken out carefully without disturbing the settled materials followed by filtering with 0.45µm filter paper and analyzing for COD. The N-content, $f_{(N)}$ and P-content, $f_{(P)}$ of the biomass were measured by collecting the mixed liquor from the reactor at the end of aerobic period and measuring TN, SN, TP, SP, and MLVSS. The following equations were used to measure the N, P-content of the biomass.

$$\text{N-content, } f_{(N)} = \left[\frac{(TN-SN)}{MLVSS} \right] \times 100\% \quad (1)$$

$$\text{P- content, } f_{(P)} = \left[\frac{(TP-SP)}{MLVSS} \right] \times 100\% \quad (2)$$

The reported P and N-content of the biomass are the average of duplicate measurements.

2.4 Simultaneous nitrification-denitrification (SND) efficiency

SND efficiency is defined (Eq.1) as the loss of nitrogen in a typical operational cycle after accounting for biomass synthesis:

$$\% \text{ SND} = [(TKN_{i,e} - sTKN_{e,e} - NO_{x-N,e} - N_{syn}) \div (TKN_{i,e} - N_{syn})] \times 100\% \quad (3)$$

Where, $TKN_{i,e}$ is the influent total Kjeldahl nitrogen (ammonia-N plus organic N) concentration (mg/L), $sTKN_{e,e}$ is the effluent soluble TKN concentration(mg/L), $NO_{x-N,e}$ is the sum of effluent nitrite-N and nitrate-N concentration(mg/L), N_{syn} is the nitrogen used (mg/L) for biomass synthesis.

SND efficiency simultaneously takes into account both nitrification and denitrification efficiency where ordinary nitrification and denitrification efficiency can be defined as follows:

$$\% \text{ Nitrification} = [(TKN_{i,e} - sTKN_{e,e} - N_{syn}) \div (TKN_{i,e} - N_{syn})] \times 100\% \quad (4)$$

$$\% \text{ Denitrification} = [(TKN_{i,e} - sTKN_{e,e} - NO_{x-N,e} - N_{syn}) \div (TKN_{i,e} - sTKN_{e,e} - N_{syn})] \times 100\% \quad (5)$$

2.5 Startup and operation of SNDPR-DBR

The SNDPR system consisted of a 2L SBR and a diffused aeration system connected to a programmable logic control (PLC) based DO controller (Appendix C, Figure S1). The operational variables of the reactor are provided in Appendix C (Table S1).

The SNDPR-SBR was inoculated with 1:1 (VSS mass basis) DPAO sludge to nitrifying sludge and operated at a DO concentration of 0.3 ± 0.05 mg/L, 15-days SRT, and 16 hr HRT i.e. 3L of wastewater was fed daily to the 2L working volume SBR. Each cycle was operated for 8 hr and consisted of 10 min feeding followed by a react period of 180 min anaerobic and 210 min aerobic. While typical anaerobic period for EBPR is about 30-60 min in the presence of enough readily biodegradable carbon for PAOs (Metcalf and Eddy, 2014), an extended anaerobic period was provided to allow for hydrolysis/fermentation of slowly biodegradable component of wastewater and subsequent P-release, particularly in the absence of enough rbCOD in the influent wastewater. The longer anaerobic period also takes into account the pre-anoxic zone that typically exists in SBR. The need for the extended anaerobic phase is justified based on the 2-phase P release discussed later. At the end of the aerobic react period, treated wastewater was settled for 70 min, followed by withdrawal of 1L water, giving a filling ratio of 50%. Room temperature was maintained between 23-25°C. The airflow was controlled at 0.1 litre/min, and the DO controller supplied the air intermittently using on/off control sequence to maintain the DO level between 0.25-0.35 mg/L. The system pH was observed to be between 7.5 and 8.1 without active control. Sludge wasting was done once a day at the end of the aerobic cycle after accounting for the effluent VSS to maintain a solids retention time (SRT) of 15 days.

3.Results and Discussions

3.1. DPAOs inoculum and wastewater characteristics

An offline batch activity test was performed in order to evaluate the anoxic P-removal activity of DPAOs inoculum from the parent-DPAOs reactor (Fig.6.1). As seen from Fig. 6.1, the majority of the VFAs were taken up by the DPAOs in the first 15 minute of the anaerobic cycle and subsequently phosphorus was released at a specific rate (SPRR) of 49 mgPO₄-P/g-VSS.h (Table 6.1). Both phosphorus and sCOD concentrations plateaued after 15 min in the anaerobic cycle signifying lack of readily biodegradable carbon for further uptake by the DPAOs. During the anoxic period, phosphorus was taken up by DPAOs along with reduction of nitrites. As seen in Fig. 6.1, in each nitrite spike period no phosphorus was taken up in the absence of nitrites, indicating that P-uptake was primarily via nitrites. During the aerobic polishing, residual phosphorus was taken up leading to complete phosphorus removal.

Table 6.1 Kinetic characteristics of DPAOs inoculum

	An ^a	Ax ^b -1	Ax-2	Ax-3	AO ^c
SPRR (mgPO₄-P/g-VSS.hr)	49	-	-	-	-
SPUR (mgPO₄-P/g-VSS.hr)	-	6.9	7.6	7.1	3.5
SDNR (mgNO₂-N /g-VSS.hr)	-	7.8	6.9	6.2	-
N-reduction rate/P-uptake rate	-	1.1	0.9	0.9	-

^aAnaerobic

^bAnoxic

^cAerobic

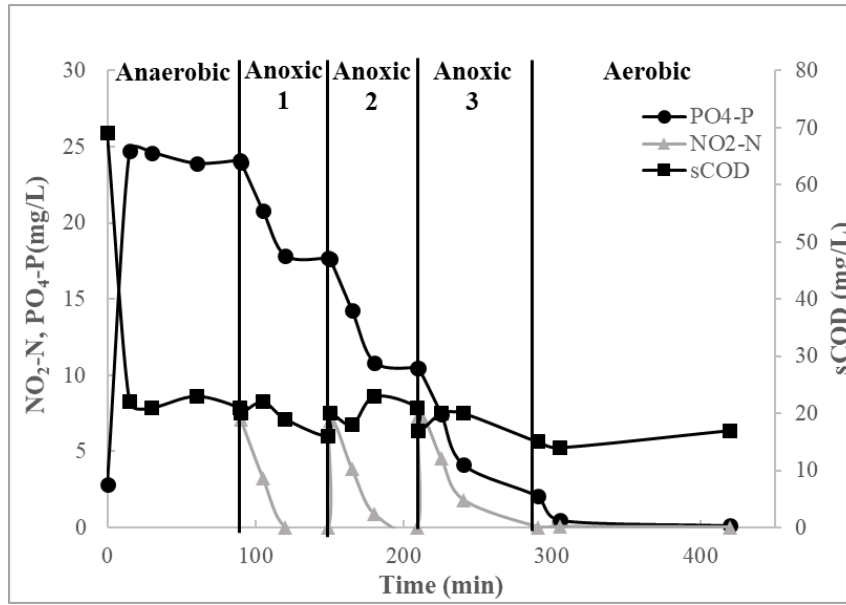


Figure 6.1 23Denitrifying P-removal activity of DPAOs inoculum

Typically, SPRR values reported in the literature, are found to be between 5 to 32 mgPO₄-P/gVSS-h (Brdjanovic *et al.*,1998; Kuba *et al.*, 1997; Mamais & Jenkins, 1992; Monti *et al.*,2007), which is lower than the SPRR obtained in this study. The literature reported SPRR values are mostly for EBPR sludge acclimatized with municipal wastewater. Kuba *et al.*(1993) reported that for enriched DPAOs culture (12.6% P-content) maximum anaerobic P-release rate was found to be between 40-60 mgPO₄-P/gVSS-h. In the current study, the P-content of the sludge in the parent DPAO reactor was found to be 11.5% of VSS by weight. Therefore, the high P-release rate signifies the successful enrichment of DPAOs culture in the parent DPAO-reactor. More than 90% of the released phosphorus was taken up by completely depleting all the added nitrites during the anoxic contact period. The specific anoxic P-uptake rates were found to be between 6.9 and 7.6 mgPO₄-P/gVSS-h (Table 6.1) which is comparable to the literature reported values of 5-20 mgPO₄-P/gVSS-h (Brdjanovic *et al.*,1998; Kuba *et al.*,1997; Mamais & Jenkins, 1992; Monti *et al.*, 2007). The average nitrogen- reduced to phosphorus-uptake rate ratio was found to be 0.98, which is lower than the reported literature values of 1.2-1.4 (Dai *et al.*,2017; Peng *et al.*, 2011).

3.2. Effluent quality and operational performance of SNDPR-SBR

To demonstrate the feasibility and evaluate the robustness of low DO SNDPR process for treating municipal wastewater, the reactor was fed with primary effluent from a municipal wastewater treatment plant. The SNDPR-SBR operated for 65 days at varying COD/N ratio from 5 to 10. A prolonged anaerobic period of 180 min was implemented to facilitate sufficient hydrolysis of carbonaceous compounds into readily biodegradable carbon and storage as internal carbon by phosphorus accumulating microorganisms. Table 6.2 shows the influent and effluent characteristics at various periods of operation of the SNDPR-SBR.

Table 6.2 Influent and effluent characteristics of SNDPR-SBR

Concentration (mg/L)	Days 1-16 (period 1)		Days 17-30 (period 2)		Days 31-38 (period 3)		Days 39-65 (period 4)	
	Influent n=3	Effluent n=6	Influent n=2	Effluent n=2	Influent n=1	Effluent n=2	Influent n=3	Effluent n=6
COD/N	7 ± 0.3		8 ± 0.04		10		5 ± 0.3	
ffCOD/TKN	3.4 ± 0.16		3.2 ± 0.2		3.3		1.4 ± 0.3	
COD	199 ± 37	36 ± 8	295 ± 40	47 ± 18	330	41 ± 4	186 ± 22	27 ± 9
sCOD	115 ± 26	21 ± 8	158 ± 45	31 ± 22	154	29 ± 2	72 ± 13	20 ± 8
ffCOD	82 ± 11		115 ± 11		108		55 ± 16	
TP	3 ± 1	0.6 ± 0.17	5 ± 1	0.7 ± 0.1	5.3	0.4 ± 0.01	3 ± 0.2	0.4 ± 0.17
SP	2 ± 1	0.2 ± 0.16	3 ± 1	0.4 ± 0.08	3.2	0.2 ± 0.02	1 ± 0.03	0.13 ± 0.10
NH ₄ -N	15 ± 5	1.5 ± 1.2	29 ± 9	2 ± 0.2	24	0.2 ± 0.07	29 ± 5	0.7 ± 0.3
NO ₃ -N	2.5 ± 1	6.4 ± 2.6	0.57 ± 0.62	1.5 ± 0.14	1.0	4.4 ± 0.07	0.1 ± 0.03	8.5 ± 1
NO ₂ -N	0.2 ± 0.02	0.1 ± 0.05	0.2 ± 0.26	0.1 ± 0.05	0.5	0.03 ± 0.003	0.2 ± 0.11	1.2 ± 0.3
TN	28 ± 6	9.5 ± 3.7	37 ± 5	4 ± 0.6	38	5 ± 0.02	39 ± 7	11 ± 0.7
SN	26 ± 7	8.4 ± 3.1	34 ± 4.7	3 ± 0.5	28	4.6 ± 0.07	33 ± 6	10 ± 0.8
TKN	26 ± 7	3 ± 1.5	36 ± 6	2.5 ± 0.5	32	0.6 ± 0.07	39 ± 7	1.6 ± 0.8
sTKN	23 ± 6	2 ± 1.4	33 ± 6	1.7 ± 0.41	26	0.2 ± 0.003	33 ± 6	0.8 ± 0.65
Alkalinity (as CaCO ₃)	317 ± 14	288 ± 50	299 ± 26	270 ± 31	303	227 ± 6	223 ± 61	140 ± 49
TSS	71 ± 13	8 ± 2.8	85 ± 18	12 ± 2.5	104	9 ± 0.7	46 ± 14	8 ± 3
VSS	51 ± 10	6 ± 1.7	68 ± 15	10 ± 2	81	6 ± 0.5	36 ± 12	6 ± 1

From day 1 to day 38, the COD/N ratio varied from 7 to 10 while the ffCOD/TKN ratio was nearly constant at around 3.3. From day 39 and onwards the COD/N ratio and ffCOD/TKN ratio were reduced to 5 and 1.4, respectively. Typically, 30% of the influent COD is ffCOD, which can be considered as readily biodegradable COD in the influent wastewater (Gupta, 2018). The inert soluble COD varied between 30-45 mg/L, which is consistent with Greenway wastewater characteristics (Gupta, 2018). While influent characteristics up to day 38 represent typical primary effluent from a municipal wastewater treatment plant, influent characteristics from day 39 represented a very challenging low strength wastewater for combined N and P removal. Fig. 6.2 shows the operational performance of SNDPR system for treating municipal wastewater. As seen in Fig. 6.2, N removal is more sensitive to COD/N compared to P-removal. Throughout the study, P-removal maintained above 90% based on average influent TP of 3, 5, 5.3, 3 PO₄-P/L and effluent SP of 0.2, 0.4, 0.2, and 0.13 mg PO₄-P/L for periods 1, 2, 3, and 4, respectively. The N-removal varied between 69% to 91% based on average influent TN of 28, 37, 38, 39 mgN/L and effluent SN of 8.4, 3, 4.6, and 10 mg N/L for periods 1, 2, 3, and 4, respectively. The ffCOD/TP ratios were 27, 23, 20, 18 mgCOD/mgPO₄-P for periods 1, 2, 3, 4, respectively (Table 6.2). The P-removal was not impacted by the decreasing trend of ffCOD/TP ratio since it was already higher than the typical values of 10-15 mgVFA-COD/mgTP (Metcalf and Eddy, 2014) required for EBPR. The ffCOD in all periods of operation was sufficient for near complete P-removal. However, the N-removal was carbon limited as the ffCOD/TKN varied between 1.4 to 3.4 (Table 6.2) representing a challenging wastewater composition for complete denitrification. Since N-removal in SNDPR is linked to P-removal, only the stoichiometric proportion of N-removal corresponding to P-removal was achieved. As COD/N ratio was increased from 7 to 10, N-removal efficiency increased from 69% to 86% (Fig. 6.2). Figs. 6.3c-6.3d show the influent and effluent nitrogen concentration as a function of COD/N ratio. Effluent NO_x-N concentration was generally found to be decreasing with increasing COD/N ratio. It can be seen from Fig. 6.3d, in spite of increased COD/N ratio, effluent NO_x-N increased from day 31-38. The increase in effluent NO_x-N in period 3 (day 31-38) compared to period 2 (day 17-30) is due to the impact of reduction in $[\text{ffCOD}_{\text{initial}} / (\text{TKN}_{\text{oxidized}} + \text{NO}_x\text{-N}_{\text{initial}})]$ ratio in the SBR. An

increase in initial $\text{NO}_x\text{-N}$ in the SBR is detrimental to the anaerobic P-release as ordinary denitrifiers competes with DPAOs for carbon. Wang *et al.*(2007) reported when initial $\text{NO}_x\text{-N}$ concentration increased from 1 to 18 mg/L , net P-release and P-release rate decreased from 12 to 0.7 mg/L and 3.3 to 0 mg $\text{PO}_4\text{P/g-VSS.h}$, respectively. The $[\text{ffCOD}_{\text{initial}}/(\text{TKN}_{\text{oxidized}}+\text{NO}_x\text{-N}_{\text{initial}})]$ ratios for periods 1, 2, 3, 4 were found to be 2.9, 4.1, 3.4, and 1.2 respectively. Although COD/N ratio increased from 8 in period 2 to 10 in period 3, $[\text{ffCOD}_{\text{initial}}/(\text{TKN}_{\text{oxidized}}+\text{NO}_x\text{-N}_{\text{initial}})]$ ratio decreased from 4.1 to 3.4. Therefore, as the $[\text{ffCOD}_{\text{initial}}/(\text{TKN}_{\text{oxidized}}+\text{NO}_x\text{-N}_{\text{initial}})]$ decreases N-removal performance of the SBR deteriorated. Therefore, it is particularly important to keep the anaerobic zone free from $\text{NO}_x\text{-N}$ for reliable EBPR performance. In full-scale systems like Johannesburg process a pre-anoxic zone is incorporated to minimize the carryover of $\text{NO}_x\text{-N}$ into the anaerobic zone. Fig.6.3e shows the influent and effluent phosphorus concentration. Irrespective of the COD/N ratio addressed in this study, the TP and SP of the effluent were always maintained around 1 and 0.5 mg/L, respectively. Throughout the operation, excellent COD and suspended solids removals were observed as evident with effluent sCOD concentration of 20-25 mg/L and suspended solids of 6-10 mg/L (Fig.6.3a-6.3b). The mixed liquor volatile solids (MLVSS) to mixed liquor suspended solids (MLSS) ratio varied between 70% to 80% (Fig.6.3f). As shown in Fig.6.3f, the average MLVSS concentration increased from 750 mg/L (days 1-16) to 1275 mg/L (days 17-30) reflecting increasing organic loading in the reactor. The reactor followed similar trend for the COD/N of 10 where the MLVSS averaged at 1535 mg/L. Between days 49 to 65, the average MLVSS was reduced to 1117 mg/L. Considering , the biomass yield of 0.27 gVSS/gCOD (determined from the linear slope of the cumulative VSS produced versus cumulative COD removed—not shown, R^2 of 0.98), SRT 15 days, HRT 16 hours, and the average COD reduction in each operational periods, the measured MLVSS were within 15%-30% of the theoretical steady state MLVSS concentration. It should be noted that, the influent COD fluctuations in this study was very dynamic and each of the operational period at the given COD/N ratio was 1 SRT turnover or less.

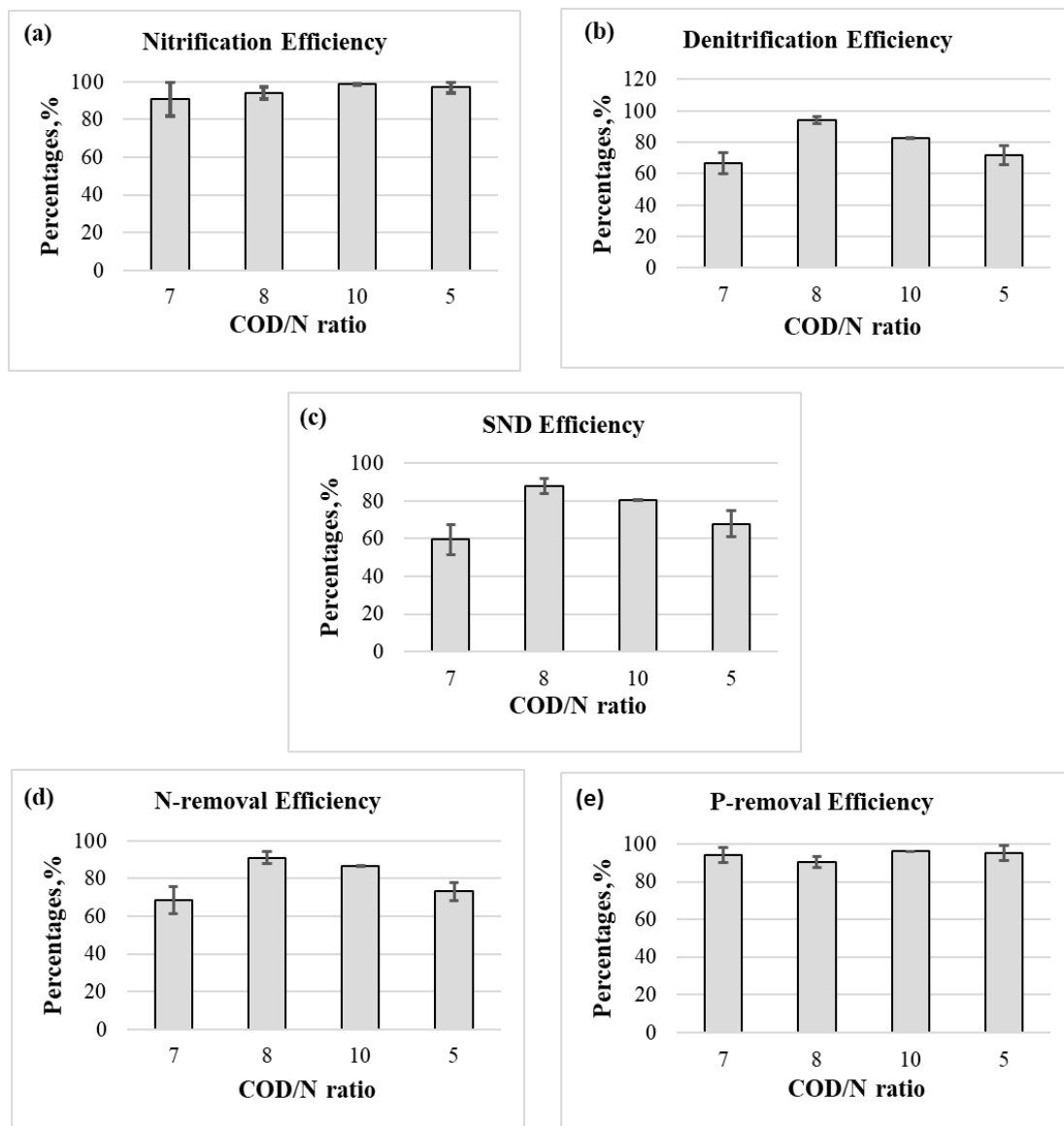


Figure 6.2 24Operational performance of SNDPR-SBR^a

a percentages are based on average influent-effluent concentrations during each period of operation at a given COD/N ratio

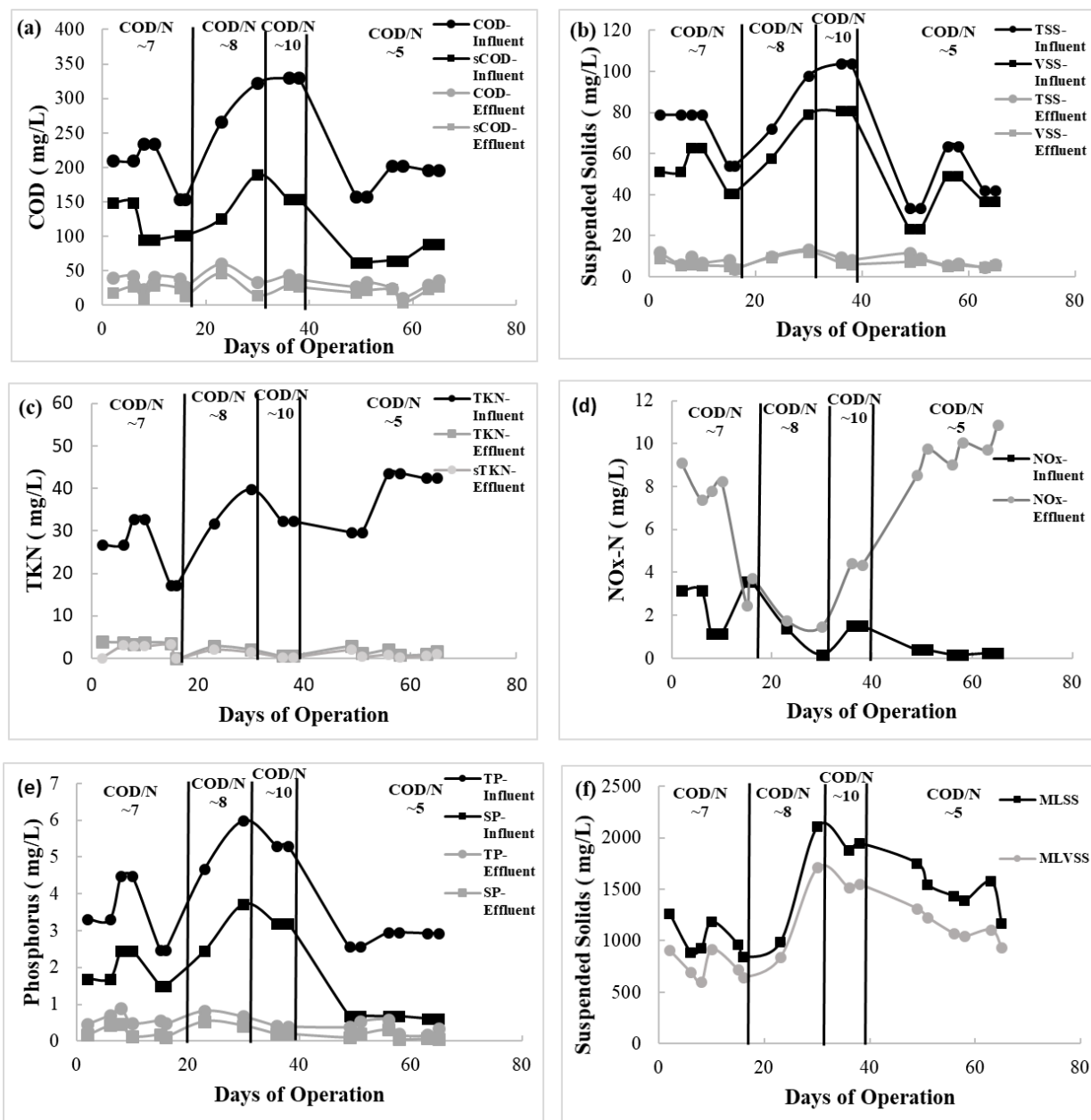


Figure 6.3 Temporal variations of influent and effluent characteristics in the SNDPR-SBR

During the entire period of reactor operation, the COD/N ratio varied between 5-10. From day1 to day16, the COD/N was 7. Average nitrogen removal efficiency was found to be about 70% with an average influent TN and effluent SN concentration of 28 and 8.4 mgN/L, respectively. Phosphorus removal efficiencies were found to be above 90% where average influent TP and effluent SP concentration were 3 and 0.2 mg PO₄-P/L, respectively. Although no supplemental carbon was provided, EBPR performance

was acceptable during this period. Average $\text{NO}_x\text{-N}$, TP, and SP concentration were 6.5, 0.60, and 0.20 mg/L, respectively (Fig. 6.3). From days 1-16, even though the influent ffCOD/TKN ratio was 3.4, the ffCOD/TP ratio was as high as 27. Therefore, the system achieved a very low SP concentration. As shown in Fig. 6.2a, nitrification efficiency during this period was as high as 92% with an average influent TKN and effluent sTKN concentration of 26 and 2 mgN/L, respectively (Fig.6.3c). Although near-complete nitrification was achieved, SND and denitrification efficiencies were 60% and 67%, respectively. As the COD/N ratio increased to 8 and then 10 between days 17- 38, average nitrogen and phosphorus removal efficiencies also improved to 90% (based on average influent TN and effluent SN of 37.5 and 3.8 mgN/L) and 94% (based on average influent TP and effluent SP of 5.15 and 0.3 mg $\text{PO}_4\text{-P/L}$), respectively. Both SND and denitrification efficiencies were found to be above 80%. During this period (days 17-38), additional COD in the influent wastewater helped to further reduce the effluent $\text{NO}_x\text{-N}$ concentration to stay between 2-4 mg/L. Even though organic loading increased during day 17 to day 48, nitrification was not impacted as evident by high nitrification efficiency of 94% based on average influent TKN of (Fig.6.2a) and low effluent TKN concentration of 0.8-2 mg/L (Fig.6.3c). During day 17-48, ffCOD/T were 20-23 mgCOD/mg $\text{PO}_4\text{-P}$ (Table 6.2) which was sufficient to maintain effluent TP and SP concentration of 0.4-0.7 mg $\text{PO}_4\text{-P/L}$ and 0.2-0.4 mg $\text{PO}_4\text{-P/L}$, respectively. From day 49 and onward, COD/N ratio was decreased to 5 to find out the performance of the SNDPR-SBR for treating carbon limited municipal wastewater. The ffCOD/TP remained high at 18 mgCOD/mg $\text{PO}_4\text{-P}$. The nitrogen and phosphorus removal remained stable without significant deterioration of removal efficiencies (Fig.6.2). During this period (days 39-65), N-removal efficiency dropped to 74%(based on average influent TN of 39 mgN/L and effluent SN 10 mgN/L), and P-removal remained as high as 95% (based on average influent TP of 3 mg $\text{PO}_4\text{-P/L}$ and effluent SP of 0.13 mg/L) . The high P-removal was primarily due to the high sufficient readily biodegradable carbon with respect to phosphorus as evidenced by the high ffCOD/TP ratio (18) in the influent wastewater. Even though both COD/N ratio (5) and ffCOD/TKN (1.4) ratio beyond day 39 were lower than the period of day 1 to day 16, denitrification efficiency did not decrease. This signifies lower carbon demand for denitrification beyond day 39. While high $\text{NO}_x\text{-N}$

concentrations as observed beyond day 39 can be troublesome for SBR operation as it gives denitrifiers a competitive advantage over PAOs/DPAOs prior to the anaerobic stage, this can be easily overcome in continuous flow systems, such as A²O, MUCT, etc, by primary sludge and/or RAS fermentation.

On day 64, an online cycle test was performed in order to determine the nutrient removal kinetics in the SNDPR-SBR. Table 6.3 gives the kinetic parameters for N and P-removal in the reactor. Fig.6.4 shows the variations in sCOD, nitrogen, and phosphorus concentration in a typical cycle of SNDPR-SBR on day 64.

Table 6.3 Kinetic parameter and operational performance of a typical cycle in SNDPR-SBR (day 64)

Parameters	SNDPR-SBR
SPRR1 (mg PO ₄ -P/g.VSS.h)	19
SPRR2 (mg PO ₄ -P/g.VSS.h)	1.6
SPUR (mg PO ₄ -P/g.VSS.h)	11
SAUR (mg NH ₄ -N/g. VSS.h)	4.9

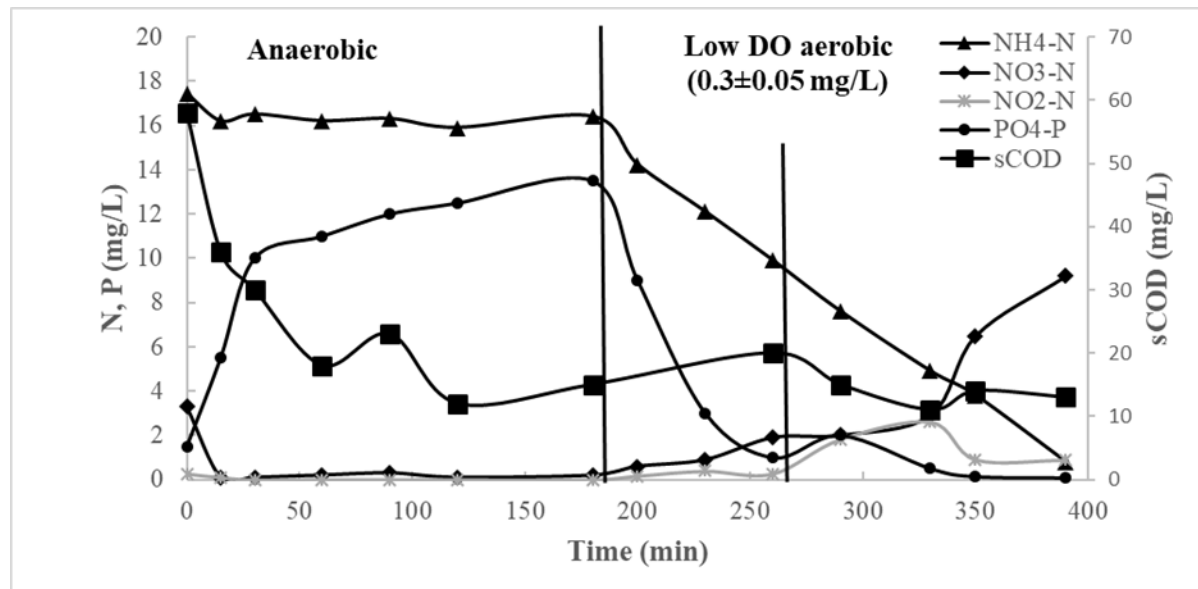


Figure 6.4 Cyclic variation of COD, N, and P in the SNDPR-SBR

The initial anaerobic sCOD, NH₄-N, NO₃-N, NO₂-N, PO₄-P concentrations were 58, 17.5, 3.3, 0.271, and 1.5 mg/L, respectively. As seen in Fig. 6.4, the initial pre-anoxic period lasted about 15 min leaving at least 165 min of active anaerobic contact time. Inorganic nitrogen concentration remained unchanged at about 17 mg/L throughout the

anaerobic cycle (Fig.6.4). This signifies the absence of bacterial growth or reproduction during the anaerobic period. sCOD reduction was accompanied by poly-P hydrolysis affecting an increase in orthophosphate concentration to about 14 mg/L.

A distinct 2-step P-release was observed. As seen in Fig.6.4, in the first 30 min in the anaerobic period a rapid P-release (19 mgPO₄-P/gVSS-h) was observed. This is well within the reported values of 5-32 mg PO₄-P/g VSS-h (Kuba et al., 1997; D. Mamais & Jenkins, 1992). The sp. substrate uptake rate during the period was 34 mg sCOD/gVSS-h. After accounting for the initial nitrate reduction consuming 14 mg sCOD/L based on a COD/NO_x-N ratio of 4.2 as determined by mass balance, the P-released to COD utilized ratio was approximately 0.55. The rapid P-release is primarily due to the assimilation of readily biodegradable carbon, which is mostly VFA or ffCOD. From 30 min to 180 min during the anaerobic period a slower P-release (1.6 mgPO₄-P/gVSS-h) was observed. While the slower P-release is well below the literature values, it should be noted that majority of the literature reported sp. uptake rate are determined using synthetic carbon source, such as acetate, propionate, and ethanol (R. P X Hesselmann et al., 2000; Adrian Oehmen et al., 2005; Puig et al., 2007; Yagci et al., 2003). The substrate uptake was also in agreement with the P-release and proceeds at a sp. substrate uptake rate of 6.73 mg sCOD/gVSS-h. The P-released to COD utilized ratio was 0.23. The slower P-release corresponds to slowly biodegradable or fermentable carbon uptake. The difference in P-release to COD uptake ratio clearly shows the difference in the nature of the carbon source utilized for P-release during the anaerobic phase. While the P-release to COD utilized in the two segments of the anaerobic period was outside the range of typical values, the overall P-released to COD utilized ratio was found to be 0.43, which is close to the reported literature values of 0.3-0.4 (Kuba *et al.*, 1993, 1997). P-release to COD utilized ratio for the first 60 min of the anaerobic period was 0.37 compared to 0.43 for 180 min. This also justifies the extension of anaerobic period to 180 min facilitating improved intracellular carbon storage.

In the subsequent aerobic phase (after 180 minutes), NH₄-N was oxidized (AUR:4.9 mgNH₄-N/gVSS-h) to nitrite/nitrate and orthophosphate was taken up via nitrite/nitrate reduction to nitrogen gas. The DO was controlled between 0.25 to 0.35 mg/L. No significant nitrite/nitrate accumulated during the first 1.5 hours in the aerobic

cycle implying the occurrence of simultaneous nitrification and denitrifying phosphorus removal. From 180 to 260 min, $\text{NO}_x\text{-N}$ produced/ $\text{NH}_4\text{-N}$ removed was found to be 0.24 while SP decreased from 13.5 to 1 mg/L and about 5 mg/L $\text{NO}_x\text{-N}$ was denitrified representing a denitrification rate of 4.2 mgN/gVSS.h. While this rate is lower than the average denitrification rate of DPAO inoculum (~ 7 mg $\text{NO}_2\text{-N}$ /gVSS.h) found in this study, it should be noted that denitrification rate in the DPAO inoculum activity test represents ideal anoxic condition compared to low DO aerobic denitrification in SNDPR reactor. Additionally, a lower SDNR compared to DPAO inoculum activity test could potentially imply that both nitrite and nitrate were reduced during 180-260 min of aerobic period. However, the majority of the biodegradable carbon was utilized in the anaerobic period and the sCOD remained unchanged during 180-260 min, implying occurrence of SND via denitrifying phosphorus removing microorganism. For post anoxic denitrification where substrate for denitrification is provided by endogenous decay, SDNR may vary from 0.63-2.5 mg $\text{NO}_3\text{-N}$ / g-VSS.hr, 0.9-4 mg $\text{NO}_2\text{-N}$ / g-VSS.hr (USEPA, 2007; Metcalf & Eddy, 2014; Yan et al., 2019). Considering, the denitrification rate of DPAOs inoculum and as observed in the cyclic test, DPAOs have competitive advantage over ordinary denitrifiers in the absence of readily biodegradable exogenous carbon. Moreover, nitrite denitrification rate of DPAOs inoculum is significantly higher compared to the post anoxic nitrite denitrification by ordinary denitrifiers implying the significance of nitrite accumulation in the reactor both in terms of outcompeting ordinary denitrifiers and carbon savings. From 260 to 390 min of the operational cycle, $\text{NO}_x\text{-N}$ produced/ $\text{NH}_4\text{-N}$ removed was found to be 0.90, which shows lack of denitrification. Only about 2 mg/L of P-removal occurred during this 130 min signifying near complete depletion of stored PHA between 180 to 260 min of the operation cycle. During the aerobic cycle, $\text{NH}_4\text{-N}$ and $\text{PO}_4\text{-P}$ concentrations decreased by 17 and 14 mg/L with effluent $\text{NO}_3\text{-N}$, $\text{NO}_2\text{-N}$ concentrations of 9.2, and 0.88 mg/L, respectively signifying a loss of 6 mg/L nitrogen after accounting for 1 mg/L nitrogen for biomass synthesis. Since sCOD remained nearly constant at 15 mg/L throughout the low DO aeration, exogenous denitrification by OHO can be ignored, and denitrification during the aerobic stage can be primarily attributed to DPAOs using endogenous carbon and nitrate/nitrite as an electron acceptor. The pH of the reactor varied between 7.5 to 8.1 throughout the period of

operation. According to (Filipe *et al.*, 2001) the anaerobic substrate utilization of GAOs are limited at and above pH of 7.25. In addition, previous studies showed that PAOs have competitive advantage over GAOs at low DO condition, as PAOs have a higher oxygen affinity and thus maintain their activity at low DO concentration, while GAO activity decreased (Canearrvalheira *et al.*, 2014; Lemaire *et al.*, 2006). Compared to DO concentration of 8 mg/L, at a DO concentration of 0.6 mg/L, P-uptake rate and PHA consumption rate of PAOs decreased by 20% and 27% , respectively, while the PHA consumption and glycogen production rates of GAOs decreased by 77% and 88%, respectively (Carvalho *et al.*, 2014). Therefore, low DO condition in the current study is highly favourable for GAO washout from the microbial consortium

During the aerobic period, a rapid P uptake (10 mgPO₄-P/gVSS-h) from 180 to 260 min period followed by a slower P-uptake (2 mgPO₄-P/gVSS-h) from 260 to 390 min was observed. The overall P-uptake rate was 11 mgPO₄-P/gVSS-h, which is well within the reported literature value of 6-21 mg PO₄-P/g VSS-h (Kuba *et al.*, 1997; Mamais & Jenkins, 1992). The ratio of P-uptake/P-release was 1.12, which is also close to the reported literature value of 1.05 to 1.10 (Pan *et al.*, 2017; Wang *et al.*, 2015). Based on P-uptake/ P-release ratio, if the anaerobic contact time was reduced to a typical value of 0.5h, a net phosphorus release of 8.5 mg/L would have been achieved. This would result in an excess phosphorus uptake of ~0.85 mg/L leaving the effluent SP and TP to be ~0.8 mg/L and ~1.1 mg/L , respectively in period 4 (day 39-65), which would not meet the effluent P-discharge limit. This further justified the extended anaerobic contact time of 180 min in this study. Ratios of P-uptake to N-reduced from 180 to 260 min and 260 to 390 min in the operation cycle were 2.9 , and 3.5 mg P/ mg N, respectively. The reported typical values for P-uptake/N-removed for DPAOs are 1 to 2 and 4 to 6 for nitrite and nitrate, respectively (Lee & Yun, 2014; Peng *et al.*, 2011). This further signifies that both nitrate and nitrite was used for P-removal during low DO aerobic period in the SNDPR system. However, lack of nitrite accumulation and lower P-uptake to N-reduced ratio during the initial aerobic period (180-260 minutes) signifies the competitive advantage of DPAOs over NOBs for nitrite consumption at low DO. This is also evident in Fig. 6.4, when P-removal was almost complete by 260 min, after which nitrate rapidly accumulated until the end of the cycle. Mehrabi et al. (2020) also reported the occurrence

of nitrite shunt due to denitrifying microorganisms outperforming NOBs in a intermittently aerated membrane aerated biofilm reactor at sCOD/N ratio of 2.5.

The results from the reactor operation at COD/N ratio 5, showed that integrating EBPR with SND via DPAOs can be advantageous for combined C, N, and P removal from carbon limited municipal wastewater. The low DO aerobic operation in this study is particularly beneficial to DPAOs for simultaneous N and P removal in comparison to high DO processes. This is due to the fact that for a given floc size, at low DO a larger anoxic core can be obtained since the oxygen transfer driving force is low and more intracellular carbon is available for anoxic P-removal rather than aerobic P-removal in the outer shell.

With respect to energy saving, low DO operation implies greater oxygen transfer driving force which means a significant reduction in aeration requirement and cost of operation. Considering a temperature of 23°C (saturation DO concentration of 8.5 mg/L) and a β (ratio of saturation DO in wastewater to clean water) of 0.95, at a DO concentration 0.3 mg/L the mass transfer driving force is 1.54 times that at 3 mg/L which translates to an approximate reduction of 35% in the air flow. Similarly, reduction of COD utilized/NO_x-N to ~ 4.5 in the current study from 6-8 in the typical nitrifying plant and no additional VFA (typically 10-15 mg VFA/mg PO₄-P applied) for P-removal also reduced carbon requirement for N and P removal. For example, in operational period 2 (day 17-30), 81 mg NO_x-N and 14 mg PO₄-P was removed per day. In conventional EBPR, it will require 626 mg COD/day (81mgNO_x-N/day×6mgCOD/NO_x-N+14 mgPO₄-P×10mgCOD/mg PO₄-P) compared to 365 mg COD/day (81mgNO_x-N/day×4.5 mgCOD/NO_x-N + 0) signifies approximately 42% saving in carbon requirement.

3.3. N-P distribution and mass balances

N and P mass balance in the SBR were performed for the period between day 39 to day 65 (COD/N ~5). Equation 6 was used to calculate the input-N to the SBR.

$$N_{inf} (mg/d) = Q \times [C_{TKN, inf} + C_{NOx-N, inf}] \quad (6)$$

Where, Q and C refer to the flow and concentration in litre/day and mg/litre, respectively.

The nitrogen in the reactor mainly consumed via two distinct reaction pathways: (1) nitrification-denitrification, and (2) biomass synthesis. The output-N from the reactor is determined from the following equations:

$$N_{eff.} (mg/d) = N_{clarified\ eff} + N_{denitrified} (N_2\ gas) + N_{waste\ sludge} \quad (7)$$

$$N_{clarified\ eff} (mg/d) = Q \times [C_{sTKN,eff} + C_{NOx-N,eff} + f_{(N)} \times C_{VSS,eff}] \quad (8)$$

$$N_{denitrified} (mg/d) = Q \times [C_{TKN,inf} - C_{sTKN,eff} - C_{N-biomass\ synthesis} - C_{NOx-N,eff}] \quad (9)$$

$$N_{waste\ sludge} (mg/d) = [C_{MLVSS} \times V_R / SRT - Q \times C_{VSS,eff}] \times f_{(N)} \quad (10)$$

Where, $f_{(N)}$, V_R , SRT represents the N-content of the biomass, reactor volume, solid retention time, respectively. SRT was maintained at 15 days for the entire duration of the study. The experimental value of $f_{(N)}$ was found to be 9.8% .

Equations 11-14 were used for performing the phosphorus mass balance. The influent phosphorus is the sum of soluble ortho-phosphorus and particulate phosphorus.

$$P_{,inf} (mg/d) = Q \times C_{TP,inf} \quad (11)$$

$$P_{,eff} (mg/d) = P_{,clarified\ eff} + P_{waste\ sludge} \quad (12)$$

$$P_{clarified\ eff} (mg/d) = Q \times [C_{SP,eff} + f_{(P)} \times C_{VSS,eff}] \quad (13)$$

$$P_{waste\ sludge} (mg/d) = [C_{MLVSS} \times V_R / SRT - Q \times C_{VSS,eff}] \times f_{(P)} \quad (14)$$

Where, $f_{(P)}$ represents the P-content of biomass, respectively. The experimental value for $f_{(P)}$ was found to be about 8.8% .

The nitrogen mass balance by considering various forms of nitrogen in the influent and effluent streams are provided in Appendix C (Table S2).

The nitrogen balance closed very well with the sum of clarified effluent-N, WAS-N, and denitrified-N accounting for about 96% of the influent total nitrogen. Fig.6.5a shows the distribution of total influent nitrogen into various effluent process streams.

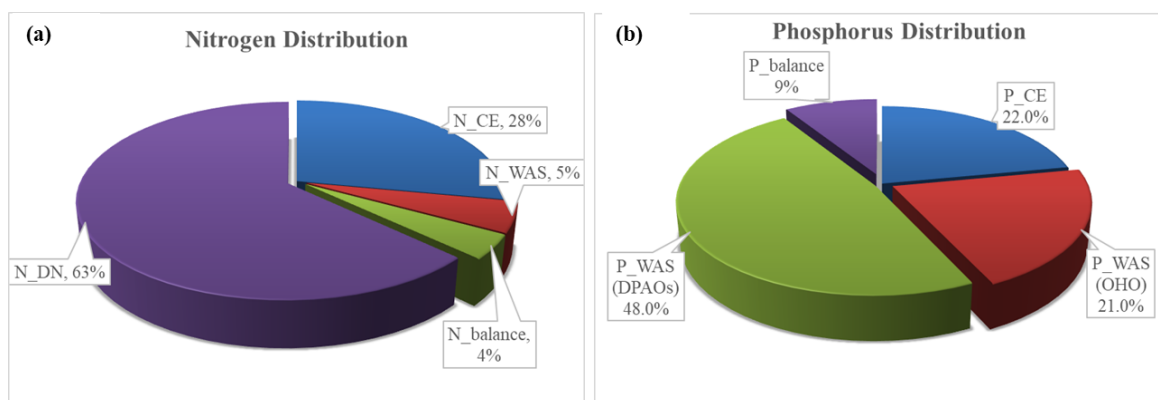


Figure 6.5 Distribution of influent nitrogen (a) and phosphorus (b) in various process streams

Approximately 28% of the influent nitrogen ended up in the clarified effluent as $\text{NO}_x\text{-N}$, TKN, and VSS-N. About 63% of the influent nitrogen was denitrified which is consistent with SND efficiency of 68%. This also signifies favourable activity of denitrifying phosphorus removing microorganisms in the system. Overall nitrogen removal efficiency was found to be 73% (Fig.6.2). Based on mass balance, about 60% of the total available COD was utilized for N-removal which signifies good carbon utilization efficiency of the system. The ratio of COD utilized to $\text{NO}_x\text{-N}$ reduced was estimated to be 4.2 implying carbon efficient denitrification. Approximately 6.5% of the influent nitrogen also partitioned in the biomass via cell synthesis and left the system with the effluent VSS and WAS.

The phosphorus content in various influent and effluent streams in the reactor are provided in Appendix C (Table S3).

The phosphorus mass balance also closed very well with the sum of effluent total phosphorus concentration in clarified effluent and waste activated sludge accounts for 91% of the influent total phosphorus. It was found that 88% of the influent phosphorus accumulated in the biomass (VSS+WAS), which clearly signifies enhanced biological phosphorus removal in the reactor (Table S3). Overall, phosphorus removal was found to be 78%, with about 69% of the influent phosphorus removed from the system with waste activated sludge and 22% with clarified effluent (Fig.6.5b). Taking into account the biomass yield of 0.27 g VSS/g COD and P-content of ordinary heterotrophs about 2%, 2.6 out of 8.6 mg $\text{PO}_4\text{-P/day}$ was removed by biomass synthesis, representing 69% of the influent phosphorus was removed by DPAOs and 31% by biomass synthesis. The P-

content of the biomass was found to be about 8.8%, which signifies a significant enrichment of PAOs or DPAOs in the activated sludge.

4. Summary and Conclusions

This study demonstrates a simultaneous nitrification and denitrifying phosphorus removal system treating municipal wastewater with moderate to low COD/N ratio at very low DO without any carbon supplementation. An extended anaerobic contact time facilitates the efficient utilization of organic carbon in wastewater and nutrient removal without carbon supplementation. Low DO during the aerobic stage was favorable for anoxic P-removal as evidenced by simultaneous N and P removal in the cyclic test. DPAOs were found to have competitive advantage over NOBs in the presence of sufficient internal carbon for denitrification. The ratio of COD utilized to $\text{NO}_x\text{-N}$ reduced was estimated to be 4.2, which also implies efficient utilization of carbon for nutrient removal. Due to the integration of nitrification with denitrifying phosphorus removal, more than 70% N-removal and 90% P-removal was observed even at low COD/N ratio of 5. Compared to the conventional EBPR process, the low DO-SNDPR process implies maximum reductions in energy and carbon consumption of 35% and 45%, respectively. This can significantly reduce the overall carbon footprint of municipal wastewater treatment plants. While the current study showed a promising approach for treating municipal wastewater minimizing carbon and oxygen consumption, impact of COD/N ratio on microbial population dynamics and biochemical modeling of DPAOs and NOBs competition for nitrite consumption under various process condition would help to further understand the low DO SNDPR process.

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Chapter 7

Conclusions and recommendations for future work

7.1 Conclusions

The overall goal of this thesis project was to investigate effective strategies for minimizing supplemental carbon usage and energy consumption. The details of the major research findings are presented in chapters 3, 4, 5, 6. A brief summary of the key outcomes from this thesis outlined below:

Municipal biosolids treated with a low-temperature thermal alkaline process (Lystek®) were investigated as an alternative carbon source for biological phosphorus removal. The performance of Lystek product was compared with synthetic VFA (60:40 acetate: propionate). In general, Lystek biosolids were found to be a suitable carbon source for phosphorus removal from municipal wastewater due to the presence of high concentrations of biodegradable soluble COD and VFAs. The extent of PAO activity largely relied on the readily biodegradable fraction of the Lystek biosolids with acetate and propionate contributing higher fraction of the soluble COD. Even though phosphorus removal kinetics were found to be one-third that of synthetic VFAs, overall nitrogen and phosphorus removal was found to be comparable to synthetic VFAs. In spite of the additional nitrogen and phosphorus contribution from Lystek biosolids, the effluent phosphorus concentrations were maintained at $TP < 1$ mg/L and $SP < 0.5$ mg/L, indicating the effectiveness of an alternative, inexpensive natural carbon source.

When simultaneous nitrification denitrification and phosphorus removal is attempted via nitrite pathway, ammonium oxidizing bacteria (AOB) and denitrifying PAOs (DPAOs) work jointly for N and P removal. Since DPAOs can also use oxygen as an electron acceptor in addition to NO_x-N , a DO limited condition might induce competition for oxygen between DPAOs and AOB. The effect of denitrifying phosphorus accumulating organisms (DPAOs) at low DO (0.5 mg/L) on simultaneous nitrification-denitrification and enhanced at a 1:1 ratio of DPAOs to nitrifying sludge did not impact nitrogen nor phosphorus removal. However, as DPAOs to nitrifiers population ratio was further increased to 4:1, the effect of low DO was found to be more significant on nitrification than P-removal. This signifies the competitive advantages of DPAOs over nitrifiers under DO-limited conditions.

Mainstream partial nitrification-denitrifying phosphorus removal was investigated at low DO (0.3 ± 0.05 mg/L) and low COD/N ratio (4) with synthetic wastewater. Partial nitrification and denitrifying phosphorus removal was found to be stable over a period of 180 days at the given conditions. Low DO and moderate SRT (15 days) was favorable for sustained DPAOs activity in the reactor with SND, N-removal, and P-removal percentages above 80%. Low DO was found to be favorable for washout of DGAOs and NOBs. The anoxic share of P-removal increased to 92%, which is significantly higher than the reported literature values (30%-50%). Also, nitrite pathway significantly reduced carbon (53%) and energy consumption (30%) compared to traditional fully nitrifying EBPR plants.

Simultaneous nitrification-denitrifying phosphorus removal was investigated for carbon limited municipal wastewater. An extended anaerobic period was found to be effective for optimal utilization of influent organic carbon. A two stage P-release was observed where the faster P-release concomitant with the utilization of readily biodegradable carbon followed by a slower release with slowly biodegradable carbon uptake. Due to optimum utilization of influent organic carbon during the anaerobic stage and active role of DPAOs at low DO, even at a low COD/N ratio of 5, N and P-removal efficiencies were maintained above 70% and 90%, respectively. Mass balances showed that the COD/NO_x-N ratio for denitrification was 4.2, which also indicates efficient utilization of carbon for nutrient removal.

7.2. Recommendations for future research

Based on the major findings of this PhD project, future research should address following topics:

- To investigate the potential for struvite precipitation from Lystek product to remove excess N and P. This will help minimize dosage requirement for Lystek product to BNR systems.
- To conduct a comprehensive molecular characterization of Lystek product and determine the extent of biodegradable carbon and inerts in Lystek
- To study the impact of low temperature ($\sim 10^{\circ}\text{C}$) and inhibitors on denitrifying biological phosphorus removal
- To investigate the impact of various operational parameters, such as DO, temperature, and SRT on the enrichment of DPAOs in the partial nitrification-denitrifying phosphorus removing systems.
- To investigate the potential of single sludge partial nitrification-annamox process operated at low DO (0.2-0.3 mg/L) and moderate to long SRT (15-25 days).

Appendices

Appendix A Supplementary information for chapter 3

Table S1a Wastewater & composite influent characteristics for reactor 1 (concentrations in mg/L)

Wastewater	COD	sCOD	VFA (as COD)	TP	SP	NH ₄ -N	NO ₃ -N	NO ₂ -N	NO _x -N	TN	sTN	TKN	TS	VSS	Alkalinity
Synthetic WW (Phase-1)	294	276	152	4.94	4.37	22.4	0.3	-	0.3	22.4	22	22.4	-	-	340
Adelaide WW (Phase 2 & 3), n=12	215 ± 27	71 ± 15	-	5.1 ± 0.6	2.9 ± 0.4	27 ± 3	0.8 ± 0.6	0.072 ± 0.132	0.8 ± 0.6	42 ± 3	33 ± 3	41 ± 3	89 ± 16	71 ± 18	312 ± 11
Composite influent (Phase 3) n=4	509 ± 29	372 ± 13	310 ± 0	4.9 ± 0.4	2.7 ± 0.3	25 ± 2	0.9 ± 0.2	0.146 ± 0.152	1 ± 0.3	41 ± 4	31 ± 4	40 ± 4	95 ± 12	68 ± 17	309 ± 15
Calumet WWTP (Phase 4) n=7	109 ± 15	34 ± 8	-	2.4 ± 0.5	1.2 ± 0.3	7 ± 2	0.5 ± 0.1	0.037 ± 0.025	0.5 ± 0.1	15 ± 3	10 ± 2	15 ± 3	66 ± 18	55 ± 14	234 ± 34
Composite influent (phase 4), n=2	246 ± 8	125 ± 8	91 ± 0	2.8 ± 0.4	1.2 ± 0.2	8 ± 2	0.5 ± 0.1	0.023 ± 0.016	0.6 ± 0.0	16 ± 3	9 ± 3	15 ± 3	76 ± 1	56 ± 11	200 ± 28

Table S1b Wastewater & composite influent characteristics for reactor 2 (concentrations in mg/L)

Wastewater	COD	sCOD	VFA (as COD)	TP	SP	NH ₄ -N	NO ₃ -N	NO ₂ -N	NO _x -N	TN	sTN	TKN	TSS	VSS	Alkalinity
Synthetic WW (Phase-1)	294	276	152	4.94	4.37	22.4	0.3	-	0.3	22.4	22	22.4	-	-	340
Adelaide WW (Phase 2 & 3), n=12	215 ± 27	71 ± 15	-	5.1 ± 0.6	2.9 ± 0.4	27 ± 3	0.8 ± 0.6	0.072 ± 0.132	0.8 ± 0.6	42 ± 3	33 ± 3	41 ± 3	89 ± 16	71 ± 18	312 ± 11
Composite influent (Phase 3), n=4	559 ± 29	372 ± 13	61* ± 12	10.1 ± 0.5	6.1 ± 0.5	34 ± 2	1.3 ± 0.2	0.146 ± 0.152	1.4 ± 0.3	73 ± 4	54 ± 4	72 ± 4	95 ± 12	68 ± 17	338 ± 15
Calumet (Phase 4) N=7	109 ± 15	34 ± 8	-	2.4 ± 0.5	1.2 ± 0.3	7 ± 2	0.5 ± 0.1	0.037 ± 0.025	0.5 ± 0.1	15 ± 3	10 ± 2	15 ± 3	66 ± 18	55 ± 14	234 ± 34
Composite influent (Phase 4), n=2	526 ± 8	352 ± 8	47* ± 4	3.8 ± 0.4	1.3 ± 0.2	25 ± 2	0.8 ± 0.1	0.039 ± 0.015	0.8 ± 0.0	54 ± 3	44 ± 3	53 ± 3	76 ± 1	56 ± 11	255 ± 28

*VFA concentration refers to acetate and propionate fraction of Lystek VFA

Table S2a Lystek biosolids and filtrate characteristics (concentrations in g/L)

	COD	sCOD	VFA	TP	SP	NH ₄ -N	TN	sTN	TSS	VSS	Alkalinity
Lystek biosolids n=2	170 ± 22	47 ± 19	37.8	3.5 ± 0.1	0.3 ± 0.4	1.0 ± 0.3	8.0 ± 0.1	3.6 ± 0.2	112 ± 27	69 ± 5	19 ± 2
Lystek Filtrate+ n=4	6.8 ± 1.7	5.9 ± 1.7	1* ± 0.4	0.069 ± 0.043	0.038 ± 0.030	0.181 ± 0.003	0.609 ± 0.133	0.427 ± 0.042	-	-	-

+ Lystek filtrate was obtained by 10 times dilution of the original Lystek biosolids followed by centrifugation (10000 r/min) and filtration (1.2µm)

* VFA concentration refers to acetate and propionate fraction only as measured by HACH TNT 872

Table S2b VFA fractionation of soluble Lystek biosolids (concentrations in g/L)

	Acetic acid	Propionic acid	Isobutyric acid	Butyric acid	Isovaleric acid	Valeric acid	Total
VFA (g/L) as COD	6.8	4.2	6.4	11.1	4.6	4.8	37.8

Mitigation of carbon source requirement via Lystek biosolids filtrate (undiluted):
Table S3a Typical medium strength domestic wastewater characterization parameters and values (Metcalf and Eddy, 2014)

Component	Concentration, mg/L
COD	508
sCOD	177
BOD	200
TSS	195
VSS	150
TKN	35
NH ₄ -N	20
NO ₃ -N	0
Total phosphorus	5.6
Alkalinity	200

Table S3b Lystek biosolids characteristics (this study)

	COD	sCOD	VFA	TP	SP	NH ₄ -N	TN	sTN	TSS	VSS	Alkalinity
Lystek biosolids n=2	170 ± 22	47 ± 19	37.8	3.5 ± 0.1	0.3 ± 0.4	1.0 ± 0.3	8.0 ± 0.1	3.6 ± 0.2	112 ± 27	69 ± 5	19 ± 2

Assumptions:

65% VSS removal in primary clarifier

Secondary treatment: biomass yield, Y= 0.45 (Metcalf and Eddy, 5th Edition, Table 7.8)

SRT=10 day

Decay coefficient=0.1

SP/TP ratio in wastewater=0.6 (based on wastewater used in this study)

VSS destruction during anaerobic digestion for primary and secondary sludge are 50% and 40%, respectively.

pCOD/VSS ratio for anaerobically digested primary and secondary sludge are 2 and 1.6, respectively.

30% particulate COD (pCOD) solubilization in Lystek reactor

Primary sludge production rate

$$150 \text{ mg/L} \times 0.65 \approx 98 \text{ mg VSS/L of WW}$$

Assuming 65% VSS reduction in primary clarifier,

$$\text{COD reduction in primary clarifier} = [1 - [(508 - 177) \times 0.35 + 177] / 508] \times 100 \approx 42\%$$

Secondary sludge production rate

$$Y_{\text{obs}} = 0.45 / (1 + 0.1 \times 10) = 0.225 \text{ g VSS/g COD}$$

$$\text{Secondary sludge production} = 0.225 \times 508 \times (1 - 0.42) \approx 65 \text{ mg VSS/L WW}$$

Combined anaerobic digestion and Lystek process

$$\text{Total pCOD to Lystek reactor} = [98 \times 0.5 \times 2] + [65 \times 0.6 \times 1.6] \approx 161 \text{ mg pCOD/LWW}$$

$$\text{Soluble COD in Lystek product} = 161 \times 0.3 \approx 49 \text{ mg sCOD/LWW}$$

From Table S2a, in Lystek product, VFA/sCOD = 0.80

$$\text{VFA production in Lystek} = 49 \times 0.80 \approx 40 \text{ mg VFA/LWW}$$

From Table S2a, in Lystek product, SP/sCOD = 0.3/47 = 0.006

Total influent soluble phosphorus (SP) = SP from wastewater + SP from Lystek filtrate

$$= 5.6 \times 0.6 + 0.006 \times 49$$

$$= 3.36 + 0.29$$

$$= 3.65 \text{ mg P/L influent}$$

Therefore, VFA to P ratio in composite (Lystek filtrate + wastewater) influent = 40/3.65 ≈

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In EBPR practice, typical values for mg VFA required/mg P removed are between 10 to 15. Therefore, Lystek biosolid filtrate should mitigate the carbon source requirement for enhanced biological phosphorus removal process in typical municipal wastewater treatment plant.

Appendix B Supplementary information for chapter 5

Table S1. N and P removal kinetics in the mother DPAO reactor

	An	Ax-1	Ax-2	Ax-3	AO
SPRR (mgPO₄-P/g-VSS.hr)	53	-	-	-	-
SPUR (mgPO₄-P/g-VSS.hr)	-	4.9	5.3	4	3.7
SDNR (mgNO₂-N /g-VSS.hr)	-	5.5	5.1	4.3	-
N-reduction rate/P-uptake rate	-	1.12	0.96	1.07	-

Table S2. Kinetic parameters and operational performance of PNDPR-SBR

Kinetic parameters and operational performance	PNDPR-SBR
SPRR (mgPO ₄ -P/g-VSS.hr)	53
SPUR (mgPO ₄ -P/g-VSS.hr)	21
SAUR (mg NH ₄ -N/g-VSS.hr)	4.7
%SND	80
%P-Removal	93
% N-Removal	77

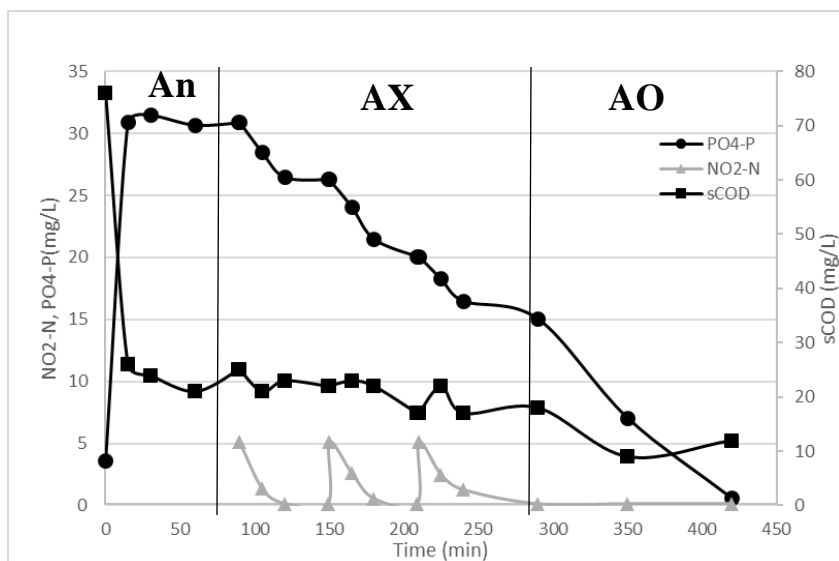


Figure S1. Operation performance of a typical cycle in the mother SBR

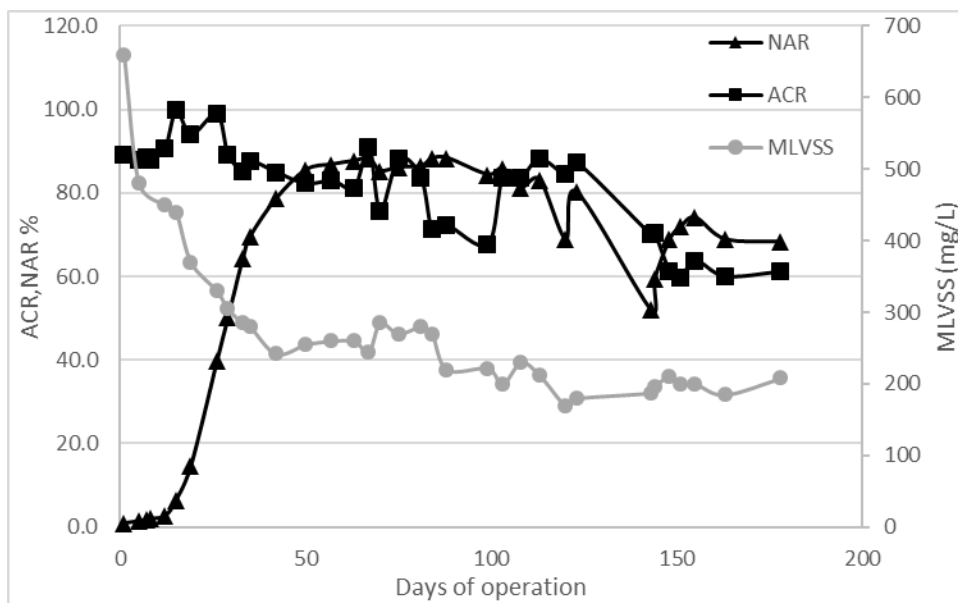


Figure S2. Nitrite accumulation in PNDPR reactor (phase 1)

a. Steady-state nitrogen and phosphorus mass balance

Nitrogen and phosphorus mass balance in the SBR were performed for the period between day 70 to day 175. Equation 1 was used to determine the input-N to the SBR.

$$\text{Influent-N (mg/d)} = Q \times [C_{\text{Inf-TKN}} + C_{\text{Inf-NO}_x}] \quad (1)$$

Where, Q and C represents the flow and concentration in litre/day and mg/litre, respectively.

The influent nitrogen to the reactor is primarily transformed via two pathways: (1) nitrification/denitrification, and (2) cell synthesis. The output-N from the reactor is calculated from following equations:

$$\text{Effluent-N (mg/d)} = N_{\text{CE}} + N_{\text{DN}} + N_{\text{WAS}} \quad (2)$$

$$N_{\text{CE}} \text{ (mg/d)} = Q \times [C_{\text{Eff-sTKN}} + C_{\text{Eff-NO}_x} + f_N \times C_{\text{Eff-VSS}}] \quad (3)$$

$$N_{\text{DN}} \text{ (mg/d)} = Q \times [C_{\text{Inf-TKN}} - C_{\text{Eff-sTKN}} - C_{\text{N-cell synthesis}} - C_{\text{Eff-NO}_x}] \quad (4)$$

$$N_{\text{WAS}} \text{ (mg/d)} = [C_{\text{MLVSS}} \times V_R / \theta_C - Q \times C_{\text{Eff-VSS}}] \times f_N \quad (5)$$

Where, N_{CE} , N_{DN} , N_{WAS} , f_N , V_R , θ_C represents the nitrogen in the clarified effluent, denitrification, waste activated sludge, N-content of the biomass, reactor volume, solid retention time, respectively. The value of θ_C was maintained at 15 days throughout the study. The value of f_N was measured experimentally and found to be between 9% to 10% at steady state condition.

Equations 6-9 were used for performing the phosphorus mass balance. The influent phosphorus is the sum of soluble ortho-phosphorus and particulate phosphorus.

$$\text{Influent-P (mg/d)} = Q \times C_{\text{Inf-TP}} \quad (6)$$

$$\text{Effluent-P (mg/d)} = P_{\text{CE}} + P_{\text{WAS}} \quad (7)$$

$$P_{\text{CE}} \text{ (mg/d)} = Q \times [C_{\text{Eff-SP}} + f_P \times C_{\text{Eff-VSS}}] \quad (8)$$

$$P_{\text{WAS}} \text{ (mg/d)} = [C_{\text{MLVSS}} \times V_R / \theta_C - Q \times C_{\text{Eff-VSS}}] \times f_P \quad (9)$$

Where, P_{CE} , P_{WAS} , f_P represents phosphorus in the clarified effluent, waste activated sludge, and the P-content of biomass, respectively. The experimental value for f_P were found to be about 15% at steady state condition (day 90).

b. Apparent specific growth rate for AOB and NOB

The apparent specific growth rate of the nitrifiers can be represented by the following formula:

$$\mu_{\text{apparent}} = \mu_{\text{max}} \left[\frac{\text{DO}}{\text{DO} + K_o} \right] \theta^{(T-\theta)} - b \theta^{(T-20)} - 1/\text{SRT}$$

Where, μ_{max} = maximum specific growth rate, g-VSS/g-VSS.day

K_o = half-velocity coefficient for DO, mg/L

b = specific endogenous decay coefficient g-VSS/g-VSS.day

θ = temperature correction coefficient

T = reactor temperature, °C

SRT = solid retention time, day

From Metcalf and Eddy (2014) page 755, following kinetic coefficients can be obtained at 20°C

Coefficients	AOB	NOB
μ_{max}	0.9	1
K_o	0.5	0.9
b	0.17	0.17
$\theta (\mu_{\text{max}})$	1.072	1.063
$\theta (b)$	1.029	1.029

At given operating condition ($T=24^\circ\text{C}$, $\text{SRT}=11$ day, $\text{DO}=0.25$ mg/L),

$$\begin{aligned} \mu_{\text{apparent,AOB}} &= 0.9 \times \left[\frac{0.25}{(0.25+0.5)} \right] 1.072^{(24-20)} - 0.17 \times 1.029^{(24-20)} - (1/11) \\ &= 0.11 \text{ d}^{-1} \end{aligned}$$

$$\begin{aligned} \mu_{\text{apparent,NOB}} &= 1 \times \left[\frac{0.25}{(0.25+0.9)} \right] 1.063^{(24-20)} - 0.17 \times 1.029^{(24-20)} - (1/11) \\ &= -0.01 \text{ d}^{-1} \end{aligned}$$

c. Contribution of N-P species to overall nutrient removal in the PNDPR system

N-Cell synthesis (mg-N/day)

$$= (\text{COD}_{\text{inf}} - \text{sCOD}_{\text{eff}}) \text{ mg/L} \times Y \times 0.1 \text{ mg-N/mg-VSS} \times 3 \text{ L/day}$$

$$= (180 - 6) \times 0.15 \times 0.1 \times 3 = 7.9 \text{ mg/day}$$

P-Cell synthesis (mg-P/day)

$$= (\text{COD}_{\text{inf}} - \text{sCOD}_{\text{eff}}) \text{ mg/L} \times Y \times 0.002 \text{ mg-P/mg-OHO} \times 3 \text{ L/day}$$

$$= (180 - 6) \times 0.15 \times 0.002 \times 3$$

$$= 2 \text{ mg/day}$$

N-Oxidized (mg-N/day)

$$= [(\text{NH}_4\text{-N}_{\text{inf}} - \text{NH}_4\text{-N}_{\text{eff}}) \text{ mg/L} - (\text{N-Cell synthesis}) \text{ mg/L}] \times 3 \text{ L/day}$$

$$= [(45 - 1) - (7.9/3)] \times 3 = 124 \text{ mg/day}$$

NO₂-N reduced by DPAOs (mg/day)

$$= \text{N}_{\text{oxidized}} \times \text{NAR (82\%)} - \text{NO}_2\text{-N}_{\text{eff}} \text{ [NAR of 82\% obtained from batch test\#1]}$$

$$= [124 \times 0.82] - (0.45 \times 3)$$

$$= 101 \text{ mg/day}$$

NO₃-N reduced by DPAOs (mg/day)

$$= \text{N}_{\text{oxidized}} \times (1 - \text{NAR}) (18\%) - \text{NO}_3\text{-N}_{\text{eff}}$$

$$= [124 \times 0.18] - (2.2 \times 3)$$

$$= 15 \text{ mg/day}$$

Net P-uptake (anoxic+aerobic) by DPAOs (mg-P/day) during low DO aeration

$$= [44 \text{ mg/L} \times 2 \text{ L} \times 3 \text{ L/day}] - \text{P}_{\text{cell synthesis}}$$

$$= 264 - 2$$

$$= 262 \text{ mg/day}$$

Net P-release (mg-P/day)

$$= \text{Net P-uptake by DPAOs} - \text{Influent P}$$

$$= (44 \text{ mg/L} \times 2 \text{ L} \times 3 \text{ L/day}) - (5 \text{ mg/L} \times 3 \text{ L/day})$$

$$= 249 \text{ mg/day}$$

P-removal via NO₂-N (mg-P/day)

$$\begin{aligned} &= \text{NO}_2\text{-N reduced} \times \Delta P / \Delta N \quad [\text{From batch test\#2 (Table 5.1), } \Delta P / \Delta N \text{ for nitrite equals 1.8}] \\ &= 101 \times 1.8 = 182 \text{ mg/day} \end{aligned}$$

P-removal via NO₃-N (mg-P/day)

$$\begin{aligned} &= \text{NO}_3\text{-N reduced} \times \Delta P / \Delta N \quad [\text{From batch test\#2 (Table 5.1), } \Delta P / \Delta N \text{ for nitrate equals 3.9}] \\ &= 15 \times 3.9 = 59 \text{ mg/day} \end{aligned}$$

P-removal via O₂ (mg-P/day)

$$\begin{aligned} &= \text{Net P-uptake by DPAOs} - \text{Anoxic P-uptake} \\ &= 262 - (182 + 59) \\ &= 21 \text{ mg/day} \end{aligned}$$

Ratio of P-uptake / P-release = 1.06

Ratio of PO₄-P removal / NO_x-N reduced via DPAOs = 241/116 = 2.08

Ratio of DPR via nitrite / DPR via nitrate = 182/59 = 3.1

Pathways for P-removal

Anoxic (NO₂-N): Anoxic (NO₃-N): Aerobic = 69% : 23% : 8%

Appendix C Supplementary information for chapter 6

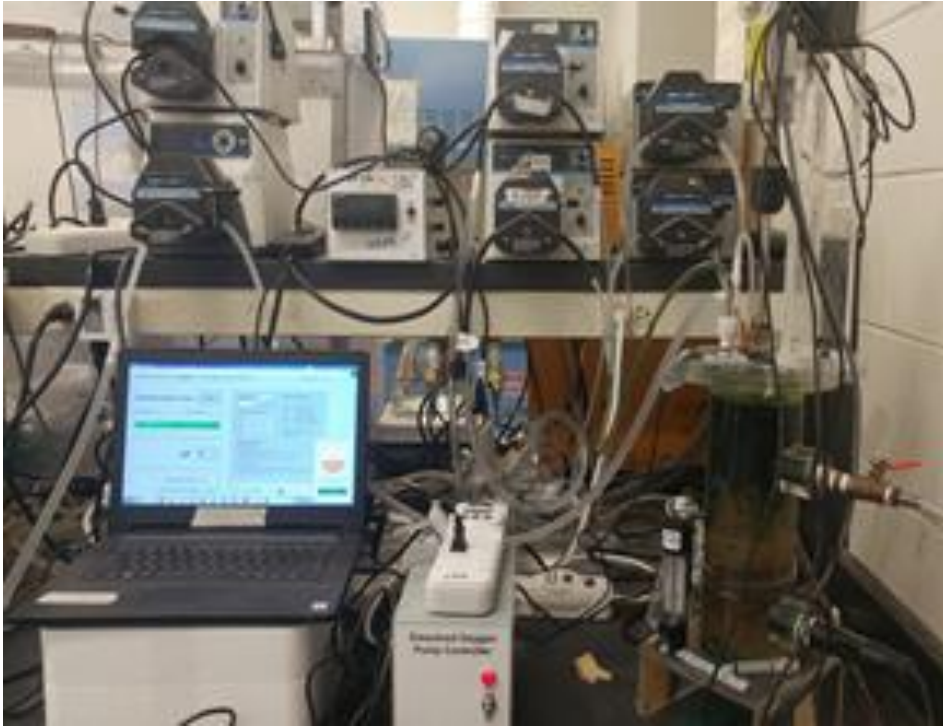


Figure S1. SNDPR-SBR system

Table S1. Operational parameters of SNDPR reactor

Parameters	Value
Working volume (L)	2
Volume exchange ratio	0.5
Cycles per day	3
SRT (day)	15
HRT (hour)	16
Air flow rate (L/min)	0.1
Sequence of operation, min (fill, anaerobic, aerobic, settle, decant)	10, 180, 210, 70, 10

Table S2. Nitrogen mass balance (mg/day)

Parameters	Input-N	Output-N
TKN	117	-
sTKN	-	2.4
NO _x -N	0.8	29
WAS-N		6
N-Denitrified		74
Effluent VSS-N		1.8
Closure (Output-N/Input-N) ×100%	96%	

Table S3. Phosphorus mass balance (mg/day)

Parameters	Influent -P	Effluent-P
Total phosphorus	8.4	
Soluble phosphorus		0.3
Phosphorus in WAS		5.8
Phosphorus in effluent VSS		1.6
Closure (Output-P/Input-P)×100 %	91 %	

Curriculum Vitae

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EDUCATION	Doctor of Philosophy-Chemical Engineering 2020 (<i>expected</i>) Western University, London, ON, Canada Thesis: <i>Biological Nutrient Removal: Minimizing Carbon and Oxygen Requirements</i> Supervisor: Dr. George Nakhla
	Master of Philosophy-Chemical Engineering 2015 University of Queensland, Brisbane, Australia Thesis: <i>Silica characterization in coal seam gas water and its removal by activated alumina</i> Supervisor(s): Dr. Steven Pratt and Dr. Greg Birkett
	Masters of Science-Chemical Engineering 2010 University of New Brunswick (UNB), Fredericton, NB, Canada CGPA: 4.3/4.3 (highest GPA in the graduating class of 2010) Thesis: <i>Cationic Surface Functionalization of Nanocrystalline Cellulose and Its Application in Textile Coating</i> Supervisor: Dr. Yonghao Ni and Dr. Huning Xiao
	Bachelor of Science-Chemical Engineering 2006 Bangladesh University of Engineering and Technology (BUET), Dhaka, Bangladesh Class Rank: 4/63 ; CGPA: 3.76/4 (degree awarded with Dean's List Honours)
PROFESSIONAL EXPERIANCE	Research Assistant 2012-2014 Advanced Water Management Center, Brisbane, Australia <ul style="list-style-type: none">• Effectively managed a multi-disciplinary research project on coal seam gas water treatment• Identified the minerals composition of scalants in reject streams in a reverse osmosis (RO) based water treatment process that led to the cost effective design of downstream wastewater processing• Developed an activated alumina (AcA) based bench-scale experimental setup and proved the ability of AcA for removal of dissolved and particulate scalants from RO brine

Visiting Scholar

2011-2012

Waterloo Institute for Nanotechnology, Waterloo, Canada

- Successfully completed several proof of concept preliminary research projects for utilization of nanocrystalline cellulose for chemical and environmental application
- Utilized modified and un-modified cellulose nanocrystals for removal of dyes from wastewater. Further work on this project received \$112,000 in funding through the federal government's Grand Challenges Canada in 2015.
- Trained and supervised summer interns for successful completion of innovative research projects
- Developed effective instrumental training procedure for research students and reduced queue in the instrumental training in the lab

Graduate Research Assistant

2008-2010

Limerick Pulp and Paper Research Center, Fredericton, Canada

- Developed and optimized the synthetic procedure for cationic functionalization of nanocrystalline cellulose (NCC)
- Formulated a NCC based coating and implemented in textile application
- Actively engaged in a number of consulting projects on pulp fiber quality improvement

PUBLICATIONS

Total Citation: 512, H-index: 6, i10- index: 6
(Google Scholar, July 10, 2020)

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HONOURS

AND

AWARDS

- NSERC-Alexander Graham Bell Canada Graduate Scholarship *2016- 2019*
- Doctoral Excellence Award-Western University *2016- 2019*
- **Western** Graduate Research Scholarship *2015- 2019*
- UQ-Origin Energy Graduate Scholarship *2012-2014*
- UNB Graduate Research Assistantship *2008 –2010*
- Merit Certificate for Academic Excellence *2003*
- University Merit Scholarship *2001-2006*
- Dean's List Award *2001-2006*